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81925 München (DE)**(54) PROCESS FOR PRODUCING L-LYSINE**

(57) The L-lysine-producing ability and the L-lysine-producing speed are improved in a coryneform bacterium harboring an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, by successively enhancing DNA coding for a dihydrodipicolinate reductase, DNA coding for a dihydrodipicolinate synthase, DNA coding for a diaminopimelate decarboxylase, and DNA coding for a diaminopimelate dehydrogenase.

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## Description

## Technical Field

5 The present invention relates to a method for producing L-lysine by cultivating a microorganism obtained by modifying a coryneform bacterium used for fermentative production of amino acid or the like by means of a technique based on genetic engineering.

## Background Art

10 L-Lysine, which is used as a fodder additive, is usually produced by a fermentative method by using an L-lysine-producing mutant strain belonging to the coryneform bacteria. Various L-lysine-producing bacteria known at present are those created by artificial mutation starting from wild type strains belonging to the coryneform bacteria.

15 As for the coryneform bacteria, there are disclosed a vector plasmid which is autonomously replicable in bacterial cells and has a drug resistance marker gene (see United States Patent No. 4,514,502), and a method for introducing a gene into bacterial cells (for example, Japanese Patent Laid-open No. 2-207791). There is also disclosed a possibility for breeding an L-threonine- or L-isoleucine-producing bacterium by using the techniques as described above (see United States Patent Nos. 4,452,890 and 4,442,208). As for breeding of an L-lysine-producing bacterium, a technique is known, in which a gene participating in L-lysine biosynthesis is incorporated into a vector plasmid to amplify the gene  
20 in bacterial cells (for example, Japanese Patent Laid-open No. 56-160997).

Known genes for L-lysine biosynthesis include, for example, a dihydrodipicolinate reductase gene (Japanese Patent Laid-open No. 7-75578) and a diaminopimelate dehydrogenase gene (Ishino, S. et al., Nucleic Acids Res., 15, 3917 (1987)) in which a gene participating in L-lysine biosynthesis is cloned, as well as a phosphoenolpyruvate carboxylase gene (Japanese Patent Laid-open No. 60-87788), a dihydrodipicolinate synthase gene (Japanese Patent Publication  
25 No. 6-55149), and a diaminopimelate decarboxylase gene (Japanese Patent Laid-open No. 60-62994) in which amplification of a gene affects L-lysine productivity.

As for enzymes participating in L-lysine biosynthesis, a case is known for an enzyme which undergoes feedback inhibition when used as a wild type. In this case, L-lysine productivity is improved by introducing an enzyme gene having such mutation that the feedback inhibition is desensitized. Those known as such a gene specifically include, for example,  
30 an aspartokinase gene (International Publication Pamphlet of WO 94/25605).

As described above, certain successful results have been obtained by means of amplification of genes for the L-lysine biosynthesis system, or introduction of mutant genes. For example, a coryneform bacterium, which harbors a mutant aspartokinase gene with desensitized concerted inhibition by lysine and threonine, produces a considerable amount of L-lysine (about 25 g/L). However, this bacterium suffers decrease in growth speed as compared with a bacterium harboring no mutant aspartokinase gene. It is also reported that L-lysine productivity is improved by further introducing a dihydrodipicolinate synthase gene in addition to a mutant aspartokinase gene (Applied and Environmental Microbiology, 57(6), 1746-1752 (1991)). However, such a bacterium suffers further decrease in growth speed.

As for the dihydrodipicolinate reductase gene, it has been demonstrated that the activity of dihydrodipicolinate reductase is increased in a coryneform bacterium into which the gene has been introduced, however, no report is included for the influence on L-lysine productivity (Japanese Patent Laid-open No. 7-75578).  
40

In the present circumstances, no case is known for the coryneform bacteria, in which anyone has succeeded in remarkable improvement in L-lysine yield without restraining growth by combining a plurality of genes for L-lysine biosynthesis. No case has been reported in which growth is intended to be improved by enhancing a gene for L-lysine biosynthesis as well.

## Disclosure of the Invention

45 An object of the present invention is to improve the L-lysine-producing ability and the growth speed of a coryneform bacterium by using genetic materials of DNA sequences each coding for aspartokinase (hereinafter referred to as "AK", provided that a gene coding for an AK protein is hereinafter referred to as "lysC", if necessary), dihydrodipicolinate reductase (hereinafter referred to as "DDPR", provided that a gene coding for a DDPR protein is hereinafter referred to as "dapB", if necessary), dihydrodipicolinate synthase (hereinafter abbreviate as "DDPS", provided that a gene coding for a DDPS protein is hereinafter referred to as "dapA", if necessary), diaminopimelate decarboxylase (hereinafter referred to as "DDC", provided that a gene coding for a DDC protein is hereinafter referred to as "lysA", if necessary),  
50 and diaminopimelate dehydrogenase (hereinafter referred to as "DDH", provided that a gene coding for a DDH protein is hereinafter referred to as "ddh", if necessary) which are important enzymes for L-lysine biosynthesis in cells of coryneform bacteria.

When an objective substance is produced fermentatively by using a microorganism, the production speed, as well

as the yield of the objective substance relative to an introduced material, is an extremely important factor. An objective substance may be produced remarkably inexpensively by increasing the production speed per a unit of fermentation equipment. Accordingly, it is industrially extremely important that the fermentative yield and the production speed are compatible with each other. The present invention proposes a solution for the problem as described above in order to fermentatively produce L-lysine by using a coryneform bacterium.

The principle of the present invention is based on the fact that the growth of a coryneform bacterium can be improved, and the L-lysine-producing speed thereof can be improved by making enhancement while combining dapB with mutant lysC (hereinafter simply referred to as "mutant lysC", if necessary) coding for mutant AK (hereinafter simply referred to as "mutant type AK", if necessary) in which concerted inhibition by lysine and threonine is desensitized, as compared with a case in which lysC is enhanced singly, and that the L-lysine-producing speed can be further improved in a stepwise manner by successively enhancing dapA, lysA, and ddh.

Namely, the present invention lies in a recombinant DNA autonomously replicable in cells of coryneform bacteria, comprising a DNA sequence coding for an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, and a DNA sequence coding for a dihydrodipicolinate reductase. The present invention provides a recombinant DNA further comprising a DNA sequence coding for a dihydrodipicolinate synthase, in addition to each of the DNA sequences described above. The present invention provides a recombinant DNA further comprising a DNA sequence coding for a diaminopimelate decarboxylase, in addition to the three DNA sequences described above. The present invention provides a recombinant DNA further comprising a DNA sequence coding for a diaminopimelate dehydrogenase, in addition to the four DNA sequences described above.

In another aspect, the present invention provides a coryneform bacterium harboring an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, and comprising enhanced DNA coding for a dihydrodipicolinate reductase. The present invention provides a coryneform bacterium further comprising enhanced DNA coding for a dihydrodipicolinate synthase in the aforementioned coryneform bacterium. The present invention provides a coryneform bacterium further comprising enhanced DNA coding for a diaminopimelate decarboxylase in the aforementioned coryneform bacterium, in addition to the three DNA's described above. The present invention provides a coryneform bacterium further comprising enhanced DNA coding for a diaminopimelate dehydrogenase in the aforementioned coryneform bacterium, in addition to the four DNA's described above.

In still another aspect, the present invention provides a method for producing L-lysine comprising the steps of cultivating any one of the coryneform bacteria described above in an appropriate medium, producing and accumulating L-lysine in a culture of the bacterium, and collecting L-lysine from the culture.

The coryneform bacteria referred to in the present invention are a group of microorganisms as defined in Bergey's Manual of Determinative Bacteriology, 8th ed., p. 599 (1974), which are aerobic Gram-positive rods having no acid resistance and no spore-forming ability. The coryneform bacteria include bacteria belonging to the genus Corynebacterium, bacteria belonging to the genus Brevibacterium having been hitherto classified into the genus Brevibacterium but united as bacteria belonging to the genus Corynebacterium at present, and bacteria belonging to the genus Brevibacterium closely relative to bacteria belonging to the genus Corynebacterium.

The present invention will be explained in detail below.

#### (1) Preparation of genes for L-lysine biosynthesis used for the present invention

The genes for L-lysine biosynthesis used in the present invention are obtained respectively by preparing chromosomal DNA from a bacterium as a DNA donor, constructing a chromosomal DNA library by using a plasmid vector or the like, selecting a strain harboring a desired gene, and recovering, from the selected strain, recombinant DNA into which the gene has been inserted. The DNA donor for the gene for L-lysine biosynthesis used in the present invention is not specifically limited provided that the desired gene for L-lysine biosynthesis expresses an enzyme protein which functions in cells of coryneform bacteria. However, the DNA donor is preferably a coryneform bacterium.

All of the genes of lysC, dapA, and dapB originating from coryneform bacteria have known sequences. Accordingly, they can be obtained by performing amplification in accordance with the polymerase chain reaction method (PCR; see White, T. J. et al., Trends Genet., 5, 185 (1989)).

Each of the genes for L-lysine biosynthesis used in the present invention is obtainable in accordance with certain methods as exemplified below.

#### (1) Preparation of mutant lysC

A DNA fragment containing mutant lysC can be prepared from a mutant strain in which synergistic feedback inhibition on the AK activity by L-lysine and L-threonine is substantially desensitized (International Publication Pamphlet of WO 94/25605). Such a mutant strain can be obtained, for example, from a group of cells originating from a wild type strain of a coryneform bacterium subjected to a mutation treatment by applying an ordinary mutation treatment such as



ultraviolet irradiation and treatment with a mutating agent such as N-methyl-N'-nitro-N-nitrosoguanidine. The AK activity can be measured by using a method described by Miyajima, R. et al. in The Journal of Biochemistry (1968), 63(2), 139-148. The most preferred as such a mutant strain is represented by an L-lysine-producing bacterium AJ3445 (FERM P-1944) derived by a mutation treatment from a wild type strain of Brevibacterium lactofermentum ATCC 13869 (having its changed present name of Corynebacterium glutamicum).

Alternatively, mutant lysC is also obtainable by an in vitro mutation treatment of plasmid DNA containing wild type lysC. In another aspect, information is specifically known on mutation to desensitize synergistic feedback inhibition on AK by L-lysine and L-threonine (International Publication Pamphlet of WO 94/25605). Accordingly, mutant lysC can be also prepared from wild type lysC on the basis of the information in accordance with, for example, the site-directed mutagenesis method.

A fragment comprising lysC can be isolated from a coryneform bacterium by preparing chromosomal DNA in accordance with, for example, a method of Saito and Miura (H. Saito and K. Miura, Biochem. Biophys. Acta, 72, 619 (1963)), and amplifying lysC in accordance with the polymerase chain reaction method (PCR; see White, T. J. et al., Trends Genet., 5, 185 (1989)).

DNA primers are exemplified by single strand DNA's of 23-mer and 21-mer having nucleotide sequences shown in SEQ ID NOs: 1 and 2 in Sequence Listing in order to amplify, for example, a region of about 1,643 bp coding for lysC based on a sequence known for Corynebacterium glutamicum (see Molecular Microbiology (1991), 5(5), 1197-1204; Mol. Gen. Genet. (1990), 224, 317-324). DNA can be synthesized in accordance with an ordinary method by using DNA synthesizer model 380B produced by Applied Biosystems and using the phosphoamidite method (see Tetrahedron Letters (1981), 22, 1859). PCR can be performed by using DNA Thermal Cycler Model PJ2000 produced by Takara Shuzo, and using Taq DNA polymerase in accordance with a method designated by the supplier.

It is preferred that lysC amplified by PCR is ligated with vector DNA autonomously replicable in cells of E. coli and/or coryneform bacteria to prepare recombinant DNA, and the recombinant DNA is introduced into cells of E. coli beforehand. Such provision makes following operations easy. The vector autonomously replicable in cells of E. coli is preferably a plasmid vector which is preferably autonomously replicable in cells of a host, including, for example, pUC19, pUC18, pBR322, pHSG299, pHSG399, pHSG398, and RSF1010.

When a DNA fragment having an ability to allow a plasmid to be autonomously replicable in coryneform bacteria is inserted into these vectors, they can be used as a so-called shuttle vector autonomously replicable in both E. coli and coryneform bacteria.

Such a shuttle vector includes the followings. Microorganisms harboring each of vectors and deposition numbers in international deposition facilities are shown in parentheses.

pHC4: Escherichia coli AJ12617 (FERM BP-3532)  
 pAJ655: Escherichia coli AJ11882 (FERM BP-136)  
Corynebacterium glutamicum SR8201 (ATCC 39135)  
 pAJ1844: Escherichia coli AJ11883 (FERM BP-137)  
Corynebacterium glutamicum SR8202 (ATCC 39136)  
 pAJ611: Escherichia coli AJ11884 (FERM BP-138)  
 pAJ3148: Corynebacterium glutamicum SR8203 (ATCC 39137)  
 pAJ440: Bacillus subtilis AJ11901 (FERM BP-140)

These vectors are obtainable from the deposited microorganisms as follows. Cells collected at a logarithmic growth phase were lysed by using lysozyme and SDS, followed by separation from a lysate by centrifugation at 30,000 x g to obtain a supernatant to which polyethylene glycol is added, followed by fractionation and purification by means of cesium chloride-ethidium bromide equilibrium density gradient centrifugation.

E. coli can be transformed by introducing a plasmid in accordance with, for example, a method of D. M. Morrison (Methods in Enzymology, 68, 326 (1979)) or a method in which recipient cells are treated with calcium chloride to increase permeability for DNA (Mandel, M. and Higa, A., J. Mol. Biol., 53, 159 (1970)).

Wild type lysC is obtained when lysC is isolated from an AK wild type strain, while mutant lysC is obtained when lysC is isolated from an AK mutant strain in accordance with the method as described above.

An example of a nucleotide sequence of a DNA fragment containing wild type lysC is shown in SEQ ID NO: 3 in Sequence Listing. An amino acid sequence of  $\alpha$ -subunit of a wild type AK protein is deduced from the nucleotide sequence, which is shown in SEQ ID NO: 4 in Sequence Listing together with the DNA sequence. Only the amino acid sequence is shown in SEQ ID NO: 5. An amino acid sequence of  $\beta$ -subunit of the wild type AK protein is deduced from the nucleotide sequence of DNA, which is shown in SEQ ID NO: 6 in Sequence Listing together with the DNA. Only the amino acid sequence is shown in SEQ ID NO: 7. In each of the subunits, GTG is used as an initiation codon, and a corresponding amino acid is represented by methionine. However, this representation refers to methionine, valine, or formylmethionine.

The mutant lysC used in the present invention is not specifically limited provided that it codes for AK in which synergistic feedback inhibition by L-lysine and L-threonine is desensitized. However, the mutant lysC is exemplified by one including mutation in which a 279th alanine residue as counted from the N-terminal is changed into an amino acid residue other than alanine and other than acidic amino acid in the  $\alpha$ -subunit, and a 30th alanine residue is changed into an amino acid residue other than alanine and other than acidic amino acid in the  $\beta$ -subunit in the amino acid sequence of the wild type AK. The amino acid sequence of the wild type AK specifically includes the amino acid sequence shown in SEQ ID NO: 5 in Sequence Listing as the  $\alpha$ -subunit, and the amino acid sequence shown in SEQ ID NO: 7 in Sequence Listing as the  $\beta$ -subunit.

Those preferred as the amino acid residue other than alanine and other than acidic amino acid include threonine, arginine, cyteine, phenylalanine, proline, serine, tyrosine, and valine residues.

The codon corresponding to an amino acid residue to be substituted is not specifically limited for its type provided that it codes for the amino acid residue. It is assumed that the amino acid sequence of possessed wild type AK may slightly differ depending on the difference in bacterial species and bacterial strains. AK's, which have mutation based on, for example, substitution, deletion, or insertion of one or more amino acid residues at one or more positions irrelevant to the enzyme activity as described above, can be also used for the present invention. Other AK's, which have mutation based on, for example, substitution, deletion, or insertion of other one or more amino acid residues, can be also used provided that no influence is substantially exerted on the AK activity, and on the desensitization of synergistic feedback inhibition by L-lysine and L-threonine.

An AJ12691 strain obtained by introducing a mutant lysC plasmid p399AK9B into an AJ12036 strain (FERM BP-734) as a wild type strain of Brevibacterium lactofermentum has been deposited on April 10, 1992 under a deposition number of FERM P-12918 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan), transferred to international deposition based on the Budapest Treaty on February 10, 1995, and deposited under a deposition number of FERM BP-4999.

## (2) Preparation of dapB

A DNA fragment containing dapB can be prepared from chromosome of a coryneform bacterium by means of PCR. The DNA donor is not specifically limited, however, it is exemplified by Brevibacterium lactofermentum ATCC 13869 strain.

A DNA sequence coding for DDPR is known for Brevibacterium lactofermentum (Journal of Bacteriology, 175(9), 2743-2749 (1993)), on the basis of which DNA primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 23-mers respectively having nucleotide sequences depicted in SEQ ID NOs: 8 and 9 in Sequence Listing. Synthesis of DNA, PCR, and preparation of a plasmid containing obtained dapB can be performed in the same manner as those for lysC described above.

A nucleotide sequence of a DNA fragment containing dapB and an amino acid sequence deduced from the nucleotide sequence are illustrated in SEQ ID NO: 10. Only the amino acid sequence is shown in SEQ ID NO: 11. In addition to DNA fragments coding for this amino acid sequence, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 11, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the DDPR activity.

A transformant strain AJ13107 obtained by introducing a plasmid pCRDAPB containing dapB obtained in Example described later on into E. coli JM109 strain has been internationally deposited since May 26, 1995 under a deposition number of FERM BP-5114 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) based on the Budapest Treaty.

## (3) Preparation of dapA

A DNA fragment containing dapA can be prepared from chromosome of a coryneform bacterium by means of PCR. The DNA donor is not specifically limited, however, it is exemplified by Brevibacterium lactofermentum ATCC 13869 strain.

A DNA sequence coding for DDPS is known for Corynebacterium glutamicum (see Nucleic Acids Research, 18(21), 6421 (1990); EMBL accession No. X53993), on the basis of which DNA primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 23-mers respectively having nucleotide sequences depicted in SEQ ID NOs: 12 and 13 in Sequence Listing. Synthesis of DNA, PCR, and preparation of a plasmid containing obtained dapA can be performed in the same manner as those for lysC described above.

A nucleotide sequence of a DNA fragment containing dapA and an amino acid sequence deduced from the nucle-

otide sequence are exemplified in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15. In addition to DNA fragments coding for this amino acid sequence, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 15, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the DDPS activity.

A transformant strain AJ13106 obtained by introducing a plasmid pCRDAPA containing dapA obtained in Example described later on into E. coli JM109 strain has been internationally deposited since May 26, 1995 under a deposition number of FERM BP-5113 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) based on the Budapest Treaty.

#### (4) Preparation of lysA

A DNA fragment containing lysA can be prepared from chromosome of a coryneform bacterium by means of PCR. The DNA donor is not specifically limited, however, it is exemplified by Brevibacterium lactofermentum ATCC 13869 strain.

In the coryneform bacteria, lysA forms an operon together with argS (arginyl-tRNA synthase gene), and lysA exists downstream from argS. Expression of lysA is regulated by a promoter existing upstream from argS (see Journal of Bacteriology, Nov., 7356-7362 (1993)). DNA sequences of these genes are known for Corynebacterium glutamicum (see Molecular Microbiology, 4(11), 1819-1830 (1990); Molecular and General Genetics, 212, 112-119 (1988)), on the basis of which DNA primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 23-mers respectively having nucleotide sequences shown in SEQ ID NO: 16 in Sequence Listing (corresponding to nucleotide numbers 11 to 33 in a nucleotide sequence described in Molecular Microbiology, 4(11), 1819-1830 (1990)) and SEQ ID NO: 17 (corresponding to nucleotide numbers 1370 to 1392 in a nucleotide sequence described in Molecular and General Genetics, 212, 112-119 (1988)). Synthesis of DNA, PCR, and preparation of a plasmid containing obtained lysA can be performed in the same manner as those for lysC described above.

In Example described later on, a DNA fragment containing a promoter, argS, and lysA was used in order to enhance lysA. However, argS is not essential for the present invention. It is allowable to use a DNA fragment in which lysA is ligated just downstream from a promoter.

A nucleotide sequence of a DNA fragment containing argS and lysA, and an amino acid sequence deduced to be encoded by the nucleotide sequence are exemplified in SEQ ID NO: 18. An example of an amino acid sequence encoded by argS is shown in SEQ ID NO: 19, and an example of an amino acid sequence encoded by lysA is shown in SEQ ID NO: 20. In addition to DNA fragments coding for these amino acid sequences, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 20, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the DDC activity.

#### (5) Preparation of ddh

A DNA fragment containing ddh can be prepared from chromosome of a coryneform bacterium by means of PCR. The DNA donor is not specifically limited, however, it is exemplified by Brevibacterium lactofermentum ATCC 13869 strain.

A DDH gene is known for Corynebacterium glutamicum (Ishino, S. et al., Nucleic Acids Res., 15, 3917 (1987)), on the basis of which primers for PCR can be prepared. Such DNA primers are specifically exemplified by DNA's of 20-mers respectively having nucleotide sequences depicted in SEQ ID NOs: 21 and 22 in Sequence Listing. Synthesis of DNA, PCR, and preparation of a plasmid containing obtained ddh can be performed in the same manner as those for lysC described above.

A nucleotide sequence of a DNA fragment containing ddh and an amino acid sequence deduced from the nucleotide sequence are illustrated in SEQ ID NO: 23. Only the amino acid sequence is shown in SEQ ID NO: 24. In addition to DNA fragments coding for this amino acid sequence, the present invention can equivalently use DNA fragments coding for amino acid sequences substantially the same as the amino acid sequence shown in SEQ ID NO: 24, namely amino acid sequences having mutation based on, for example, substitution, deletion, or insertion of one or more amino acids provided that there is no substantial influence on the DDH activity.

#### (2) Recombinant DNA and coryneform bacterium of the present invention

The coryneform bacterium of the present invention harbors an aspartokinase (mutant AK) in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, wherein DNA (dapB) coding for a dihydrodipicolinate



reductase is enhanced. In a preferred embodiment, the coryneform bacterium of the present invention is a coryneform bacterium in which DNA (dapA) coding for dihydrodipicolinate synthase is further enhanced. In a more preferred embodiment, the coryneform bacterium of the present invention is a coryneform bacterium in which DNA (lysA) coding for diaminopimelate decarboxylase is further enhanced. In a more preferred embodiment, the coryneform bacterium of the present invention is a coryneform bacterium in which DNA (ddh) coding for diaminopimelate dehydrogenase is further enhanced.

The term "enhance" DNA herein refers to the fact that the intracellular activity of an enzyme encoded by the DNA is raised by, for example, increasing the copy number of a gene, using a strong promoter, using a gene coding for an enzyme having a high specific activity, or combining these means.

The coryneform bacterium harboring the mutant AK may be those which produce the mutant aspartokinase as a result of mutation, or those which are transformed by introducing mutant lysC.

Examples of the coryneform bacterium used to introduce the DNA described above include, for example, the following lysine-producing wild type strains:

Corynebacterium acetoacidophilum ATCC 13870;  
Corynebacterium acetoglutamicum ATCC 15806;  
Corynebacterium callunae ATCC 15991;  
Corynebacterium glutamicum ATCC 13032;  
(Brevibacterium divaricatum) ATCC 14020;  
(Brevibacterium lactofermentum) ATCC 13869;  
(Corynebacterium lilium) ATCC 15990;  
(Brevibacterium flavum) ATCC 14067;  
Corynebacterium melassecola ATCC 17965;  
Brevibacterium saccharolyticum ATCC 14066;  
Brevibacterium immariophilum ATCC 14068;  
Brevibacterium roseum ATCC 13825;  
Brevibacterium thiogenitalis ATCC 19240;  
Microbacterium ammoniophilum ATCC 15354;  
Corynebacterium thermoaminogenes AJ12340 (FERM BP-1539).

Other than the bacterial strains described above, those usable as a host include, for example, mutant strains having an L-lysine-producing ability derived from the aforementioned strains. Such artificial mutant strains includes the followings: S-(2-aminoethyl)-cysteine (hereinafter abbreviated as "AEC") resistant mutant strains (Brevibacterium lactofermentum AJ11082 (NRRL B-1147), Japanese Patent Publication Nos. 56-1914, 56-1915, 57-14157, 57-14158, 57-30474, 58-10075, 59-4993, 61-35840, 62-24074, 62-36673, 5-11958, 7-112437, and 7-112438); mutant strains which require amino acid such as L-homoserine for their growth (Japanese Patent Publication Nos. 48-28078 and 56-6499); mutant strains which exhibit resistance to AEC and require amino acids such as L-leucine, L-homoserine, L-proline, L-serine, L-arginine, L-alanine, and L-valine (United States Patent Nos. 3,708,395 and 3,825,472); L-lysine-producing mutant strains which exhibit resistance to DL- $\alpha$ -amino- $\epsilon$ -caprolactam,  $\alpha$ -amino-lauryllactam, aspartate-analog, sulfa drug, quinoid, and N-lauroylleucine; L-lysine-producing mutant strains which exhibit resistance to inhibitors of oxaloacetate decarboxylase or respiratory system enzymes (Japanese Patent Laid-open Nos. 50-53588, 50-31093, 52-102498, 53-9394, 53-86089, 55-9783, 55-9759, 56-32995 and 56-39778, and Japanese Patent Publication Nos. 53-43591 and 53-1833); L-lysine-producing mutant strains which require inositol or acetic acid (Japanese Patent Laid-open Nos. 55-9784 and 56-8692); L-lysine-producing mutant strains which exhibit sensitivity to fluoropyruvic acid or temperature not less than 34 °C (Japanese Patent Laid-open Nos. 55-9783 and 53-86090); and producing mutant strains belonging to the genus Brevibacterium or Corynebacterium which exhibit resistance to ethylene glycol and produce L-lysine (United States Patent No. 4,411,997).

In a specified embodiment, in order to enhance the genes for L-lysine biosynthesis in the host as described above, the genes are introduced into the host by using a plasmid vector, transposon or phage vector or the like. Upon the introduction, it is expected to make enhancement to some extent even by using a low copy type vector. However, it is preferred to use a multiple copy type vector. Such a vector includes, for example, plasmid vectors, pAJ655, pAJ1844, pAJ611, pAJ3148, and pAJ440 described above. Besides, transposons derived from coryneform bacteria are described in International Publication Pamphlets of WO02/02627 and WO93/18151, European Patent Publication No. 445385, Japanese Patent Laid-open No. 6-46867, Vertes, A. A. et al., Mol. Microbiol., 11, 739-746 (1994), Bonamy, C., et al., Mol. Microbiol., 14, 571-581 (1994), Vertes, A. A. et al., Mol. Gen. Genet., 245, 397-405 (1994), Jagar, W. et al., FEMS Microbiology Letters, 126, 1-6 (1995), Japanese Patent Laid-open No. 7-107976, Japanese Patent Laid-open No. 7-327680 and the like.

In the present invention, it is not indispensable that the mutant lysC is necessarily enhanced. It is allowable to use

those which have mutation on lysC on chromosomal DNA, or in which the mutant lysC is incorporated into chromosomal DNA. Alternatively, the mutant lysC may be introduced by using a plasmid vector. On the other hand, dapA, dapB, lysA, and ddh are preferably enhanced in order to efficiently produce L-lysine.

Each of the genes of lysC, dapA, dapB, lysA, and ddh may be successively introduced into the host by using different vectors respectively. Alternatively, two, three, four, or five species of the genes may be introduced together by using a single vector. When different vectors are used, the genes may be introduced in any order, however, it is preferred to use vectors which have a stable sharing and harboring mechanism in the host, and which are capable of co-existing with each other.

A coryneform bacterium harboring the mutant AK and further comprising enhanced dapB is obtained, for example, by introducing, into a host coryneform bacterium, a recombinant DNA containing mutant lysC and dapB autonomously replicable in cells of coryneform bacteria.

A coryneform bacterium further comprising enhanced dapA in addition to mutant lysC and dapB is obtained, for example, by introducing, into a host coryneform bacterium, a recombinant DNA containing mutant lysC, dapB, and dapA autonomously replicable in cells of coryneform bacteria.

A coryneform bacterium further comprising enhanced lysA in addition to mutant lysC, dapB, and dapA is obtained, for example, by introducing, into a host coryneform bacterium, a recombinant DNA containing mutant lysC, dapB, dapA, and lysA autonomously replicable in cells of coryneform bacteria.

A coryneform bacterium further comprising enhanced ddh in addition to mutant lysC, dapB, dapA, and lysA is obtained, for example, by introducing, into a host coryneform bacterium, a recombinant DNA containing mutant lysC, dapB, dapA, lysA, and ddh autonomously replicable in cells of coryneform bacteria.

The above-mentioned recombinant DNAs can be obtained, for example, by inserting each of the genes participating in L-lysine biosynthesis into a vector such as plasmid vector, transposon or phage vector as described above.

In the case in which a plasmid is used as a vector, the recombinant DNA can be introduced into the host in accordance with an electric pulse method (Sugimoto et al., Japanese Patent Laid-open No. 2-207791). Amplification of a gene using transposon can be performed by introducing a plasmid which carrying a transposon into the host cell and inducing transposition of the transposon.

### (3) Method for producing L-lysine

L-Lysine can be efficiently produced by cultivating, in an appropriate medium, the coryneform bacterium comprising the enhanced genes for L-lysine biosynthesis as described above, producing and accumulating L-lysine in a culture of the bacterium, and collecting L-lysine from the culture.

The medium to be used is exemplified by an ordinary medium containing a carbon source, a nitrogen source, inorganic ions, and optionally other organic components.

As the carbon source, it is possible to use sugars such as glucose, fructose, sucrose, molasses, and starch hydrolysate; and organic acids such as fumaric acid, citric acid, and succinic acid.

As the nitrogen source, it is possible to use inorganic ammonium salts such as ammonium sulfate, ammonium chloride, and ammonium phosphate; organic nitrogen such as soybean hydrolysate; ammonia gas; and aqueous ammonia.

As organic trace nutrient sources, it is desirable to contain required substances such as vitamin B<sub>1</sub> and L-homoserine or yeast extract or the like in appropriate amounts. Other than the above, potassium phosphate, magnesium sulfate, iron ion, manganese ion and so on are added in small amounts, if necessary.

Cultivation is preferably carried out under an aerobic condition for about 30 to 90 hours. The cultivation temperature is preferably controlled at 25 ° C to 37 ° C, and pH is preferably controlled at 5 to 8 during cultivation. Inorganic or organic, acidic or alkaline substances, or ammonia gas or the like can be used for pH adjustment. L-lysine can be collected from a culture by combining an ordinary ion exchange resin method, a precipitation method, and other known methods.

### Brief Description of the Drawings

Fig. 1 illustrates a process of construction of plasmids p399AKYB and p399AK9B comprising mutant lysC.

Fig. 2 illustrates a process of construction of a plasmid pDPRB comprising dapB and Brevi.-ori.

Fig. 3 illustrates a process of construction of a plasmid pDPSB comprising dapA and Brevi.-ori.

Fig. 4 illustrates a process of construction of a plasmid p299LYSA comprising lysA.

Fig. 5 illustrates a process of construction of a plasmid pLYSAB comprising lysA and Brevi.-ori.

Fig. 6 illustrates a process of construction of a plasmid pPK4D comprising ddh and Brevi.-ori.

Fig. 7 illustrates a process of construction of a plasmid pCRCAB comprising lysC, dapB and Brevi.-ori.

Fig. 8 illustrates a process of construction of a plasmid pCB comprising mutant lysC, dapB, and Brevi.-ori.

Fig. 9 illustrates a process of construction of a plasmid pAB comprising dapA, dapB and Brevi.-ori.



Fig. 10 illustrates a process of construction of a plasmid p399DL comprising ddh and lysA.

Fig. 11 illustrates a process of construction of a plasmid pDL comprising ddh, lysA and Brevi.-ori.

Fig. 12 illustrates a process of construction of a plasmid pCAB comprising mutant lysC, dapA, dapB, and Brevi.-ori.

Fig. 13 illustrates a process of construction of a plasmid pCABL comprising mutant lysC, dapA, dapB, lysA, and Brevi.-ori.

Fig. 14 illustrates a process of construction of a plasmid pCABDL comprising mutant lysC, dapA, dapB, ddh, lysA, and Brevi.-ori.

#### Description of Preferred Embodiments

The present invention will be more specifically explained below with reference to Examples.

#### Example 1: Preparation of Wild Type lysC Gene and Mutant lysC Gene from Brevibacterium lactofermentum

##### (1) Preparation of wild type and mutant lysC's and preparation of plasmids containing them

A strain of Brevibacterium lactofermentum ATCC 13869, and an L-lysine-producing mutant strain AJ3445 (FERM P-1944) obtained from the ATCC 13869 strain by a mutation treatment were used as chromosomal DNA donors. The AJ3445 strain had been subjected to mutation so that lysC was changed to involve substantial desensitization from concerted inhibition by lysine and threonine (Journal of Biochemistry, 68, 701-710 (1970)).

A DNA fragment containing lysC was amplified from chromosomal DNA in accordance with the PCR method (polymerase chain reaction; see White, T. J. et al., Trends Genet., 5, 185 (1989)). As for DNA primers used for amplification, single strand DNA's of 23-mer and 21-mer having nucleotide sequences shown in SEQ ID NOs: 1 and 2 were synthesized in order to amplify a region of about 1,643 bp coding for lysC on the basis of a sequence known for Corynebacterium glutamicum (see Molecular Microbiology (1991), 5(5), 1197-1204; and Mol. Gen. Genet. (1990), 224, 317-324). DNA was synthesized in accordance with an ordinary method by using DNA synthesizer model 380B produced by Applied Biosystems and using the phosphoamidite method (see Tetrahedron Letters (1901), 22, 1859).

The gene was amplified by PCR by using DNA Thermal Cycler Model PJ2000 produced by Takara Shuzo, and using Taq DNA polymerase in accordance with a method designated by the supplier. An amplified gene fragment of 1,643 kb was confirmed by agarose gel electrophoresis. After that, the fragment excised from the gel was purified in accordance with an ordinary method, and it was digested with restriction enzymes NruI (produced by Takara Shuzo) and EcoRI (produced by Takara Shuzo).

pHSG399 (see Takeshita, S. et al., Gene (1987), 61, 63-74) was used as a cloning vector for the gene fragment. pHSG399 was digested with restriction enzymes SmaI (produced by Takara Shuzo) and EcoRI, and it was ligated with the amplified lysC fragment. DNA was ligated by using DNA ligation kit (produced by Takara Shuzo) in accordance with a designated method. Thus plasmids were prepared, in which the lysC fragments amplified from chromosomes of Brevibacterium lactofermentum were ligated with pHSG399 respectively. A plasmid comprising lysC from ATCC 13869 (wild type strain) was designated as p399AKY, and a plasmid comprising lysC from AJ3463 (L-lysine-producing bacterium) was designated as p399AK9.

A DNA fragment (hereinafter referred to as "Brevi.-ori") having an ability to make a plasmid autonomously replicable in bacteria belonging to the genus Corynebacterium was introduced into p399AKY and p399AK9 respectively to prepare plasmids carrying lysC autonomously replicable in bacteria belonging to the genus Corynebacterium. Brevi.-ori was prepared from a plasmid vector pHK4 containing Brevi.-ori and autonomously replicable in cells of both Escherichia coli and bacteria belonging to the genus Corynebacterium. pHK4 was constructed by digesting pHC4 with KpnI (produced by Takara Shuzo) and BamHI (produced by Takara Shuzo), extracting a Brevi.-ori fragment, and ligating it with pHSG298 having been also digested with KpnI and BamHI (see Japanese Patent Laid-open No. 5-7491). pHK4 gives kanamycin resistance to a host. Escherichia coli harboring pHK4 was designated as Escherichia coli AJ13136, and deposited on August 1, 1995 under a deposition number of FERM BP-5186 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan).

pHK4 was digested with restriction enzymes KpnI and BamHI, and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated BamHI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only BamHI. This plasmid was digested with BamHI, and the generated Brevi.-ori DNA fragment was ligated with p399AKY and p399AK9 having been also digested with BamHI respectively to prepare plasmids each containing the lysC gene autonomously replicable in bacteria belonging to the genus Corynebacterium.

A plasmid containing the wild type lysC gene originating from p399AKY was designated as p399AKYB, and a plas-

mid containing the mutant lysC gene originating from p399AK9 was designated as p399AK9B. The process of construction of p399AK9B and p399AKYB is shown in Fig. 1. A strain AJ12691 obtained by introducing the mutant lysC plasmid p399AK9B into a wild type strain of Brevibacterium lactofermentum (AJ12036 strain, FERM BP-734) was deposited on April 10, 1992 under a deposition number of FERM P-12918 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan), transferred to international deposition based on the Budapest Treaty on February 10, 1995, and deposited under a deposition number of FERM BP-4999.

## (2) Determination of nucleotide sequences of wild type lysC and mutant lysC from Brevibacterium lactofermentum

The plasmid p399AKY containing the wild type lysC and the plasmid p399AK9 containing the mutant lysC were prepared from the respective transformants to determine nucleotide sequences of the wild type and mutant lysC's. Nucleotide sequence determination was performed in accordance with a method of Sanger et al. (for example, F. Sanger et al., Proc. Natl. Acad. Sci., 74, 5463 (1977)).

The nucleotide sequence of wild type lysC encoded by p399AKY is shown in SEQ ID NO: 3 in Sequence Listing. On the other hand, the nucleotide sequence of mutant lysC encoded by p399AK9 had only mutation of one nucleotide such that 1051th G was changed into A in SEQ ID NO: 3 as compared with wild type lysC. It is known that lysC of Corynebacterium glutamicum has two subunits ( $\alpha$ ,  $\beta$ ) encoded in an identical reading frame on an identical DNA strand (see Kalinowski, J. et al., Molecular Microbiology (1991) 5(5), 1197-1204). Judging from homology, it is assumed that the gene sequenced herein also has two subunits ( $\alpha$ ,  $\beta$ ) encoded in an identical reading frame on an identical DNA strand.

An amino acid sequence of the  $\alpha$ -subunit of the wild type AK protein deduced from the nucleotide sequence of DNA is shown in SEQ ID NO: 4 together with the DNA sequence. Only the amino acid sequence is shown in SEQ ID NO: 5. An amino acid sequence of the  $\beta$ -subunit of the wild type AK protein deduced from the nucleotide sequence of DNA is shown in SEQ ID NO: 6 together with DNA. Only the amino acid sequence is shown in SEQ ID NO: 7. In each of the subunits, GTG is used as an initiation codon, and a corresponding amino acid is represented by methionine. However, this representation refers to methionine, valine, or formylmethionine.

On the other hand, mutation on the sequence of mutant lysC means occurrence of amino acid residue substitution such that a 279th alanine residue of the  $\alpha$ -subunit is changed into a threonine residue, and a 30th alanine residue of the  $\beta$ -subunit is changed into a threonine residue in the amino acid sequence of the wild type AK protein (SEQ ID NOs: 5, 7).

## Example 2: Preparation of dapB from Brevibacterium lactofermentum

### (1) Preparation of dapB and construction of plasmid containing dapB

A wild type strain of Brevibacterium lactofermentum ATCC 13869 was used as a chromosomal DNA donor. Chromosomal DNA was prepared from the ATCC 13869 strain in accordance with an ordinary method. A DNA fragment containing dapB was amplified from the chromosomal DNA in accordance with PCR. As for DNA primers used for amplification, DNA's of 23-mers having nucleotide sequences depicted in SEQ ID NOs: 8 and 9 in Sequence Listing respectively were synthesized in order to amplify a region of about 2.0 kb coding for DDPR on the basis of a sequence known for Brevibacterium lactofermentum (see Journal of Bacteriology, 157(9), 2743-2749 (1993)). Synthesis of DNA and PCR were performed in the same manner as described in Example 1. pCR-Script (produced by Invitrogen) was used as a cloning vector for the amplified gene fragment of 2,001 bp, which was ligated with the amplified dapB fragment. Thus a plasmid was constructed, in which the dapB fragment of 2,001 bp amplified from chromosome of Brevibacterium lactofermentum was ligated with pCR-Script. The plasmid obtained as described above, which had dapB originating from ATCC 13869, was designated as pCRDAPB. A transformant strain AJ13107 obtained by introducing pCRDAPB into E. coli JM109 strain has been internationally deposited since May 26, 1995 under a deposition number of FERM BP-5114 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) based on the Budapest Treaty.

A fragment of 1,101 bp containing a structural gene of DDPR was extracted by digesting pCRDAPB with EcoRV and SphI. This fragment was ligated with pHSG399 having been digested with HincII and SphI to prepare a plasmid. The prepared plasmid was designated as p399DPR.

Brevi.-ori was introduced into the prepared p399DPR to construct a plasmid carrying dapB autonomously replicable in coryneform bacteria. pHK4 was digested with a restriction enzyme KpnI (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated BamHI linker (pro-

duced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only BamHI. This plasmid was digested with BamHI, and the generated Brevi.-ori DNA fragment was ligated with p399DPR having been also digested with BamHI to prepare a plasmid containing dapB autonomously replicable in coryneform bacteria. The prepared plasmid was designated as pDPRB. The process of construction of pDPRB is shown in Fig. 2.

## (2) Determination of nucleotide sequence of dapB from Brevibacterium lactofermentum

Plasmid DNA was prepared from the AJ13107 strain harboring p399DPR, and its nucleotide sequence was determined in the same manner as described in Example 1. A determined nucleotide sequence and an amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 10. Only the amino acid sequence is shown in SEQ ID NO: 11.

## Example 3: Preparation of dapA from Brevibacterium lactofermentum

### (1) Preparation of dapA and construction of plasmid containing dapA

A wild type strain of Brevibacterium lactofermentum ATCC 13869 was used as a chromosomal DNA donor. Chromosomal DNA was prepared from the ATCC 13869 strain in accordance with an ordinary method. A DNA fragment containing dapA was amplified from the chromosomal DNA in accordance with PCR. As for DNA primers used for amplification, DNA's of 20-mers having nucleotide sequences shown in SEQ ID NOs: 12 and 13 in Sequence Listing respectively were synthesized in order to amplify a region of about 1.5 kb coding for DDPS on the basis of a sequence known for Corynebacterium glutamicum (see Nucleic Acids Research, 18(21), 6421 (1990); EMBL accession No. X53993). Synthesis of DNA and PCR were performed in the same manner as described in Example 1. pCR1000 (produced by Invitrogen, see Bio/Technology, 9, 657-663 (1991)) was used as a cloning vector for the amplified gene fragment of 1,411 bp, which was ligated with the amplified dapA fragment. Ligation of DNA was performed by using DNA ligation kit (produced by Takara Shuzo) in accordance with a designated method. Thus a plasmid was constructed, in which the dapA fragment of 1,411 bp amplified from chromosome of Brevibacterium lactofermentum was ligated with pCR1000. The plasmid obtained as described above, which had dapA originating from ATCC 13869, was designated as pCRDAPA.

A transformant strain AJ13106 obtained by introducing pCRDAPA into E. coli JM109 strain has been internationally deposited since May 26, 1995 under a deposition number of FERM BP-5113 in National Institute of Bioscience and Human Technology of Agency of Industrial Science and Technology of Ministry of International Trade and Industry (postal code: 305, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) based on the Budapest Treaty.

Brevi.-ori was introduced into the prepared pCRDAPA to construct a plasmid carrying dapA autonomously replicable in coryneform bacteria. pHK4 was digested with restriction enzymes KpnI and BamHI (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated SmaI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only SmaI. This plasmid was digested with SmaI, and the generated Brevi.-ori DNA fragment was ligated with pCRDAPA having been also digested with SmaI to prepare a plasmid containing dapA autonomously replicable in coryneform bacteria. This plasmid was designated as pDPSB. The process of construction of pDPSB(Km') is shown in Fig. 3.

## (2) Determination of nucleotide sequence of dapA from Brevibacterium lactofermentum

Plasmid DNA was prepared from the AJ13106 strain harboring pCRDAPA, and its nucleotide sequence was determined in the same manner as described in Example 1. A determined nucleotide sequence and an amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15.

## Example 4: Preparation of lysA from Brevibacterium lactofermentum

### (1) Preparation of lysA and construction of plasmid containing lysA

A wild type strain of Brevibacterium lactofermentum ATCC 13869 was used as a chromosomal DNA donor. Chromosomal DNA was prepared from the ATCC 13869 strain in accordance with an ordinary method. A DNA fragment containing argS, lysA, and a promoter of an operon containing them was amplified from the chromosomal DNA in



accordance with PCR. As for DNA primers used for amplification, synthetic DNA's of 23-mers having nucleotide sequences depicted in SEQ ID NOs: 16 and 17 in Sequence Listing respectively were used in order to amplify a region of about 3.6 kb coding for arginyl-tRNA synthase and DDC on the basis of a sequence known for *Corynebacterium glutamicum* (see *Molecular Microbiology*, 4(11), 1819-1830 (1990); *Molecular and General Genetics*, 212, 112-119 (1988)). Synthesis of DNA and PCR were performed in the same manner as described in Example 1. pHSG399 was used as a cloning vector for the amplified gene fragment of 3,579 bp. pHSG399 was digested with a restriction enzyme *Sma*I (produced by Takara Shuzo), which was ligated with the DNA fragment containing amplified *lysA*. A plasmid obtained as described above, which had *lysA* originating from ATCC 13869, was designated as p399LYSA.

A DNA fragment containing *lysA* was extracted by digesting p399LYSA with *Kpn*I (produced by Takara Shuzo) and *Bam*HI (produced by Takara Shuzo). This DNA fragment was ligated with pHSG299 having been digested with *Kpn*I and *Bam*HI. An obtained plasmid was designated as p299LYSA. The process of construction of p299LYSA is shown in Fig. 4.

*Brevi*.-ori was introduced into the obtained p299LYSA to construct a plasmid carrying *lysA* autonomously replicable in coryneform bacteria. pHK4 was digested with restriction enzymes *Kpn*I and *Bam*HI, and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated *Kpn*I linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the *Brevi*.-ori portion might be excised from pHK4 by digestion with only *Kpn*I. This plasmid was digested with *Kpn*I, and the generated *Brevi*.-ori DNA fragment was ligated with p299LYSA having been also digested with *Kpn*I to prepare a plasmid containing *lysA* autonomously replicable in coryneform bacteria. The prepared plasmid was designated as pLYSAB. The process of construction of pLYSAB is shown in Fig. 5.

## (2) Determination of nucleotide sequence of *lysA* from *Brevibacterium lactofermentum*

Plasmid DNA of p299LYSA was prepared, and its nucleotide sequence was determined in the same manner as described in Example 1. A determined nucleotide sequence and an amino acid sequence deduced to be encoded by the nucleotide sequence are shown in SEQ ID NO: 18. Concerning the nucleotide sequence, an amino acid sequence encoded by *argS* and an amino acid sequence encoded by *lysA* are shown in SEQ ID NOs: 19 and 20 respectively.

## Example 5: Preparation of *ddh* from *Brevibacterium lactofermentum*

A *ddh* gene was obtained by amplifying the *ddh* gene from chromosomal DNA of *Brevibacterium lactofermentum* ATCC 13869 in accordance with the PCR method by using two oligonucleotide primers (SEQ ID NOs: 21, 22) prepared on the basis of a known nucleotide sequence of a *ddh* gene of *Corynebacterium glutamicum* (Ishino, S. et al., *Nucleic Acids Res.*, 15, 3917 (1987)). An obtained amplified DNA fragment was digested with *Eco*T22I and *Ava*I, and cleaved edges were blunt-ended. After that, the fragment was inserted into a *Sma*I site of pMW119 to obtain a plasmid pDDH.

Next, pDDH was digested with *Sal*I and *Eco*RI, followed by blunt end formation. After that, an obtained fragment was ligated with pUC18 having been digested with *Sma*I. A plasmid thus obtained was designated as pUC18DDH.

*Brevi*.-ori was introduced into pUC18DDH to construct a plasmid carrying *ddh* autonomously replicable in coryneform bacteria. pHK4 was digested with restriction enzymes *Kpn*I and *Bam*HI, and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated *Pst*I linker (produced by Takara Shuzo) was ligated so that it was inserted into a *Pst*I site of pHSG299. A plasmid constructed as described above was designated as pPK4. Next, pUC18DDH was digested with *Xba*I and *Kpn*I, and a generated fragment was ligated with pPK4 having been digested with *Kpn*I and *Xba*I. Thus a plasmid containing *ddh* autonomously replicable in coryneform bacteria was constructed. This plasmid was designated as pPK4D. The process of construction of pPK4D is shown in Fig. 6.

## Example 6: Construction of Plasmid Comprising Combination of Mutant *lysC* and *dapA*

A plasmid comprising mutant *lysC*, *dapA*, and replication origin of coryneform bacteria was constructed from the plasmid pCRDAPA comprising *dapA* and the plasmid p399AK9B comprising mutant *lysC* and *Brevi*.-ori. p399AK9B was completely degraded with *Sal*I, and then it was blunt-ended, with which an *Eco*RI linker was ligated to construct a plasmid in which the *Sal*I site was modified into an *Eco*RI site. The obtained plasmid was designated as p399AK9BSE. The mutant *lysC* and *Brevi*.-ori were excised as one fragment by partially degrading p399AK9BSE with *Eco*RI. This fragment was ligated with pCRDAPA having been digested with *Eco*RI. An obtained plasmid was designated as pCRCAB. This plasmid is autonomously replicable in *E. coli* and coryneform bacteria, and it gives kanamycin resistance to a host, the plasmid comprising a combination of mutant *lysC* and *dapA*. The process of construction of pCRCAB is shown in Fig. 7.

Example 7: Construction of Plasmid Comprising Combination of Mutant *lysC* and *dapB*

A plasmid comprising mutant *lysC* and *dapB* was constructed from the plasmid p399AK9 having mutant *lysC* and the plasmid p399DPR having *dapB*. A fragment of 1,101 bp containing a structural gene of DDPR was extracted by digesting p399DPR with *EcoRV* and *SphI*. This fragment was ligated with p399AK9 having been digested with *Sall* and then blunt-ended and having been further digested with *SphI* to construct a plasmid comprising a combination of mutant *lysC* and *dapB*. This plasmid was designated as p399AKDDPR.

Next, Brevi.-ori was introduced into the obtained p399AKDDPR. The plasmid pHK4 containing Brevi.-ori was digested with a restriction enzyme *KpnI* (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated *BamHI* linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only *BamHI*. This plasmid was digested with *BamHI*, and the generated Brevi.-ori DNA fragment was ligated with p399AKDDPR having been also digested with *BamHI* to construct a plasmid containing mutant *lysC* and *dapB* autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pCB. The process of construction of pCB is shown in Fig. 8.

Example 8: Construction of Plasmid Comprising Combination of *dapA* and *dapB*

The plasmid pCRDAPA comprising *dapA* was digested with *KpnI* and *EcoRI* to extract a DNA fragment containing *dapA* which was ligated with the vector plasmid pHSG399 having been digested with *KpnI* and *EcoRI*. An obtained plasmid was designated as p399DPS.

On the other hand, the plasmid pCRDAPB comprising *dapB* was digested with *SacII* and *EcoRI* to extract a DNA fragment of 2.0 kb containing a region coding for DDPR which was ligated with p399DPS having been digested with *SacII* and *EcoRI* to construct a plasmid comprising a combination of *dapA* and *dapB*. The obtained plasmid was designated as p399AB.

Next, Brevi.-ori was introduced into p399AB. pHK4 containing Brevi.-ori was digested with a restriction enzyme *BamHI* (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated *KpnI* linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only *KpnI*. This plasmid was digested with *KpnI*, and the generated Brevi.-ori DNA fragment was ligated with p399AB having been also digested with *KpnI* to construct a plasmid containing *dapA* and *dapB* autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pAB. The process of construction of pAB is shown in Fig. 9.

Example 9: Construction of Plasmid Comprising Combination of *ddh* and *lysA*

The plasmid pUC18DDH comprising *ddh* was digested with *EcoRI* and *XbaI* to extract a DNA fragment containing *ddh*. This *ddh* fragment was ligated with the plasmid p399LYSA comprising *lysA* having been digested with *BamHI* and *XbaI* with cleaved edges having been blunt-ended after the digestion. An obtained plasmid was designated as p399DL. The process of construction of p399DL is shown in Fig. 10.

Next, Brevi.-ori was introduced into p399DL. pHK4 was digested with *XbaI* and *BamHI*, and cleaved edges were blunt-ended. After the blunt end formation, a phosphorylated *XbaI* linker was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only *XbaI*. This plasmid was digested with *XbaI*, and the generated Brevi.-ori DNA fragment was ligated with p399DL having been also digested with *XbaI* to construct a plasmid containing *ddh* and *lysA* autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pDL. The process of construction of pDL is shown in Fig. 11.

Example 10: Construction of Plasmid Comprising Combination of Mutant *lysC*, *dapA*, and *dapB*

p399DPS was degraded with *EcoRI* and *SphI* to form blunt ends followed by extraction of a *dapA* gene fragment. This fragment was ligated with the p399AK9 having been digested with *Sall* and blunt-ended to construct a plasmid p399CA in which mutant *lysC* and *dapA* co-existed.

The plasmid pCRDAPB comprising *dapB* was digested with *EcoRI* and blunt-ended, followed by digestion with *SacI* to extract a DNA fragment of 2.0 kb comprising *dapB*. The plasmid p399CA comprising *dapA* and mutant *lysC* was digested with *SpeI* and blunt-ended, which was thereafter digested with *SacI* and ligated with the extracted *dapB* fragment to obtain a plasmid comprising mutant *lysC*, *dapA*, and *dapB*. This plasmid was designated as p399CAB.

Next, Brevi.-ori was introduced into p399CAB. The plasmid pHK4 comprising Brevi.-ori was digested with a restric-

tion enzyme BamHI (produced by Takara Shuzo), and cleaved edges were blunt-ended. Blunt end formation was performed by using DNA Blunting kit (produced by Takara Shuzo) in accordance with a designated method. After the blunt end formation, a phosphorylated KpnI linker (produced by Takara Shuzo) was ligated to make modification so that the DNA fragment corresponding to the Brevi.-ori portion might be excised from pHK4 by digestion with only KpnI. This plasmid was digested with KpnI, and the generated Brevi.-ori DNA fragment was ligated with p399CAB having been also digested with KpnI to construct a plasmid comprising a combination of mutant lysC, dapA, and dapB autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pCAB. The process of construction of pCAB is shown in Fig. 12.

#### Example 11: Construction of Plasmid Comprising Combination of Mutant lysC, dapA, dapB, and lysA

The plasmid p299LYSA comprising lysA was digested with KpnI and BamHI and blunt-ended, and then a lysA gene fragment was extracted. This fragment was ligated with pCAB having been digested with HpaI (produced by Takara Shuzo) and blunt-ended to construct a plasmid comprising a combination of mutant lysC, dapA, dapB, and lysA autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pCABL. The process of construction of pCABL is shown in Fig. 13. It is noted that the lysA gene fragment is inserted into a HpaI site in a DNA fragment containing the dapB gene in pCABL, however, the HpaI site is located upstream from a promoter for the dapB gene (nucleotide numbers 611 to 616 in SEQ ID NO: 10), and the dapB gene is not decoupled.

#### Example 12: Construction of Plasmid Comprising Combination of Mutant lysC, dapA, dapB, ddh, and lysA

pHSG299 was digested with XbaI and KpnI, which was ligated with p399DL comprising ddh and lysA having been digested with XbaI and KpnI. A constructed plasmid was designated as p299DL. p299DL was digested with XbaI and KpnI and blunt-ended. After the blunt end formation, a DNA fragment comprising ddh and lysA was extracted. This DNA fragment was ligated with the plasmid pCAB comprising the combination of mutant lysC, dapA, and dapB having been digested with HpaI and blunt-ended to construct a plasmid comprising a combination of mutant lysC, dapA, dapB, lysA and ddh autonomously replicable in coryneform bacteria. The constructed plasmid was designated as pCABDL. The process of construction of pCABDL is shown in Fig. 14.

#### Example 13: Introduction of Plasmids Comprising Genes for L-Lysine Biosynthesis into L-Lysine-Producing Bacterium of Brevibacterium lactofermentum

The plasmids comprising the genes for L-lysine biosynthesis constructed as described above, namely p399AK9B(Cm<sup>r</sup>), pDPSB(Km<sup>r</sup>), pDPRB(Cm<sup>r</sup>), pLYSAB(Cm<sup>r</sup>), pPK4D(Cm<sup>r</sup>), pCRCAB(Km<sup>r</sup>), pAB(Cm<sup>r</sup>), pCB(Cm<sup>r</sup>), pDL(Cm<sup>r</sup>), pCAB(Cm<sup>r</sup>), pCABL(Cm<sup>r</sup>), and pCABDL(Cm<sup>r</sup>) were introduced into an L-lysine-producing bacterium AJ11082 (NRRL B-11470) of Brevibacterium lactofermentum respectively. AJ11082 strain has a property of AEC resistance. The plasmids were introduced in accordance with an electric pulse method (Sugimoto et al., Japanese Patent Laid-open No. 2-207791). Transformants were selected based on drug resistance markers possessed by the respective plasmids. Transformants were selected on a complete medium containing 5 µg/ml of chloramphenicol when a plasmid comprising a chloramphenicol resistance gene was introduced, or transformants were selected on a complete medium containing 25 µg/ml of kanamycin when a plasmid comprising a kanamycin resistance gene was introduced.

#### Example 14: Production of L-Lysine

Each of the transformants obtained in Example 13 was cultivated in an L-lysine-producing medium to evaluate its L-lysine productivity. The L-lysine-producing medium had the following composition.

##### [L-Lysine-producing medium]

The following components other than calcium carbonate (per 1 L) were dissolved to make adjustment at pH 8.0 with KOH. The medium was sterilized at 115 °C for 15 minutes, to which calcium carbonate (50 g) having been separately sterilized in hot air in a dry state was thereafter added.

Glucose	100 g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	55 g



(continued)

KH <sub>2</sub> PO <sub>4</sub>	1 g
MgSO <sub>4</sub> • 7H <sub>2</sub> O	1 g
Biotin	500 µg
Thiamin	2000 µg
FeSO <sub>4</sub> • 7H <sub>2</sub> O	0.01 g
MnSO <sub>4</sub> • 7H <sub>2</sub> O	0.01 g
Nicotinamide	5 mg
Protein hydrolysate (Mamenou)	30 ml
Calcium carbonate	50 g

Each of the various types of the transformants and the parent strain was inoculated to the medium having the composition described above to perform cultivation at 31.5 °C with reciprocating shaking. The amount of produced L-lysine after 40 or 72 hours of cultivation, and the growth after 72 hours (OD<sub>562</sub>) are shown in Table 1. In the table, lysC<sup>\*</sup> represents mutant lysC. The growth was quantitatively determined by measuring OD at 560 nm after 101-fold dilution.

Table 1

Accumulation of L-Lysine after Cultivation for 40 or 72 Hours				
Bacterial strain /plasmid	Introduced gene	Amount of produced L-lysine(g/L)		Growth (OD <sub>562</sub> /101)
		after 40 hrs	after 72 hrs	
AJ11082		22.0	29.8	0.450
AJ11082/p399AK9B	<u>lysC</u> <sup>*</sup>	16.8	34.5	0.398
AJ11082/pDPSB	<u>dapA</u>	18.7	33.8	0.410
AJ11082/pDRB	<u>dapB</u>	19.9	29.9	0.445
AJ11082/pLYSAB	<u>lysA</u>	19.8	32.5	0.356
AJ11082/pPK4D	<u>ddh</u>	19.0	33.4	0.330
AJ11082/pCRCAB	<u>lysC</u> <sup>*</sup> , <u>dapA</u>	19.7	36.5	0.360
AJ11082/pAB	<u>dapA</u> , <u>dapB</u>	19.0	34.8	0.390
AJ11082/pAB	<u>dapA</u> , <u>dapB</u>	19.0	34.8	0.390
AJ11082/pCB	<u>lysC</u> <sup>*</sup> , <u>dapB</u>	23.3	35.0	0.440
AJ11082/pDL	<u>ddh</u> , <u>lysA</u>	23.3	31.6	0.440
AJ11082/pCAB	<u>lysC</u> <sup>*</sup> , <u>dapA</u> , <u>dapB</u>	23.0	45.0	0.425
AJ11082/pCABL	<u>lysC</u> <sup>*</sup> , <u>dapA</u> , <u>dapB</u> , <u>lysA</u>	26.2	46.5	0.379
AJ11082/pCABDL	<u>lysC</u> <sup>*</sup> , <u>dapA</u> , <u>dapB</u> , <u>lysA</u> , <u>ddh</u>	26.5	47.0	0.409

As shown in Table 1, when mutant lysC, dapA, or dapB was enhanced singly, the amount of produced L-lysine was larger than or equivalent to that produced by the parent strain after 72 hours of cultivation, however, the amount of produced L-lysine was smaller than that produced by the parent strain after 40 hours of cultivation. Namely, the L-lysine-producing speed was lowered in cultivation for a short period. Similarly, when mutant lysC and dapA, or dapA and dapB were enhanced in combination, the amount of produced L-lysine was larger than that produced by the parent strain after 72 hours of cultivation, however, the amount of produced L-lysine was smaller than that produced by the parent strain after 40 hours of cultivation. Thus the L-lysine-producing speed was lowered.

On the other hand, when lysA or ddh was enhanced singly, or when lysA and ddh were enhanced in combination,

the amount of produced L-lysine was larger than that produced by the parent strain after 40 hours of cultivation, however, the amount of produced L-lysine was consequently smaller than that produced by the parent strain after the long period of cultivation because of decrease in growth.

On the contrary, in the case of the strain in which dapB was enhanced together with mutant lysC, the growth was improved, the L-lysine-producing speed was successfully restored in the short period of cultivation, and the accumulated amount of L-lysine was also improved in the long period of cultivation. In the case of the strain in which three of mutant lysC, dapA, and dapB were simultaneously enhanced, the L-lysine productivity was further improved. Both of the L-lysine-producing speed and the amount of accumulated L-lysine were improved in a stepwise manner by successively enhancing lysA and ddh.

#### Industrial Applicability

According to the present invention, the L-lysine-producing ability of coryneform bacteria can be improved, and the growth speed can be also improved.

The L-lysine-producing speed can be improved, and the productivity can be also improved in coryneform L-lysine-producing bacteria by enhancing dapB together with mutant lysC. The L-lysine-producing speed and the productivity can be further improved by successively enhancing dapA, lysA, and ddh in addition to the aforementioned genes.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: AJINOMOTO CO., INC.
- (ii) TITLE OF INVENTION: METHOD OF PRODUCING L-LYSINE
- (iii) NUMBER OF SEQUENCES: 24
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE:
  - (B) STREET:
  - (C) CITY:
  - (E) COUNTRY:
  - (F) ZIP:
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: JP 7-140614
  - (B) FILING DATE: 07-JUL-1995
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME:
  - (B) REGISTRATION NUMBER:
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE:
  - (B) TELEFAX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 bases
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
  - (A) DESCRIPTION: /desc = "synthetic DNA"
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  
 TOGCGAAGTA GCAOCTGTCA CTT

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:

23



- (A) LENGTH: 21 bases  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other..synthetic DNA

(A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACGGAATTCA ATCTTAAGGC C

21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1643 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Brevibacterium lactofermentum

(B) STRAIN: ATCC 13869

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCGCGAAGTA	GCAOCTGTCA	CTTTTGTCTC	AAATATTAAA	TCGAATATCA	ATATACGGTC	60
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GCAGAAAGAA	AACACTOCTC	TGGCTAGGTA	GACACAGTTT	ATAAAGGTAG	AGTTGAGCGG	180
GTAACCTGTC	GCAOCTAGAT	CGAAAGGTGC	ACAAAGGTGG	CCCTGGTGGT	ACAGAAATAT	240
GGGGGTTOCT	CGCTTGAGAG	TGOGGAAOCG	ATTAGAAACG	TOGCTGAACG	GATCGTTGOC	300
ACCAAGAAGG	CTGGAAATGA	TGTOGTGGTT	GTCTGCTCOG	CAATGGGAGA	CAOCCACGGAT	360
GAACCTCTAG	AACCTGCAGC	GGCAGTGAAT	CCCGTTCCGC	CAGCTCGTGA	AATGGATATG	420
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GGGGCAGAAG	CTCAATCTTT	CACTGGCTCT	CAGGCTGGTG	TGCTCAOCAC	CGAGCGCCAC	540
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 GGOGAAGACG AAGCOGTGCT TTATGCAGGC ACOGGAOGCT AAAGTTTTAA AGGAGTAGTT 1500  
 TTACAATGAC CACCATOGCA GTTGTTGGTG CAACCGGCCA GGTCGGOCAG GTTATGCGCA 1560  
 CCTTTTGGGA AGAGOGCAAT TTOCCAGCTG ACACTGTTTG TTTCTTTGCT TCCCCGCGTT 1620  
 CCGCAGGCGG TAAGATTGAA TTC 1643

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1643 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Brevibacterium lactofermentum  
 (B) STRAIN: ATCC 13869

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 217..1482

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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 Met Ala Leu Val Val Gln  
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 Lys Tyr Gly Gly Ser Ser Leu Glu Ser Ala Glu Arg Ile Arg Asn Val  
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 GCT GAA OGG ATC GTT GOC AOC AAG AAG GCT GGA AAT GAT GTC GTG GTT 330  
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 345 350 355  
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 375 380 385 390  
 GAA GAT GAT CTG GAT GCT GCT GCA CGT GCA TTG CAT GAG CAG TTC CAG 1434  
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 Leu Gly Gly Glu Asp Glu Ala Val Val Tyr Ala Gly Thr Gly Arg  
 410 415 420  
 AGTTTAAAG GAGTAGTTT ACAATGACCA CCATOGCAGT TGTTGGTGCA ACOGGCCAGG 1542  
 20 TOGGCCAGGT TATGOGCAOC CTTTGGGAAG AGCGCAATTT CCCAGCTGAC ACTGTTGTTT 1602  
 TCTTTGCTTC CCOGCGTTCC GCAGGCOGTA AGATTGAATT C 1643

## (2) INFORMATION FOR SEQ ID NO:5:

### (i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 421 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

### (ii) MOLECULE TYPE: protein

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

30 Met Ala Leu Val Val Gln Lys Tyr Gly Gly Ser Ser Leu Glu Ser Ala  
 1 5 10 15  
 Glu Arg Ile Arg Asn Val Ala Glu Arg Ile Val Ala Thr Lys Lys Ala  
 20 25 30  
 35 Gly Asn Asp Val Val Val Val Cys Ser Ala Met Gly Asp Thr Thr Asp  
 35 40 45  
 Glu Leu Leu Glu Leu Ala Ala Ala Val Asn Pro Val Pro Pro Ala Arg  
 50 55 60  
 Glu Met Asp Met Leu Leu Thr Ala Gly Glu Arg Ile Ser Asn Ala Leu  
 40 65 70 75 80  
 Val Ala Met Ala Ile Glu Ser Leu Gly Ala Glu Ala Gln Ser Phe Thr  
 85 90 95  
 Gly Ser Gln Ala Gly Val Leu Thr Thr Glu Arg His Gly Asn Ala Arg  
 100 105 110  
 45 Ile Val Asp Val Thr Pro Gly Arg Val Arg Glu Ala Leu Asp Glu Gly  
 115 120 125  
 Lys Ile Cys Ile Val Ala Gly Phe Gln Gly Val Asn Lys Glu Thr Arg  
 130 135 140  
 50 Asp Val Thr Thr Leu Gly Arg Gly Gly Ser Asp Thr Thr Ala Val Ala

55

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145                                      150                                      155                                      160  
 Leu Ala Ala Ala Leu Asn Ala Asp Val Cys Glu Ile Tyr Ser Asp Val  
 5                                      165                                      170                                      175  
 Asp Gly Val Tyr Thr Ala Asp Pro Arg Ile Val Pro Asn Ala Gln Lys  
 180                                      185                                      190  
 Leu Glu Lys Leu Ser Phe Glu Glu Met Leu Glu Leu Ala Ala Val Gly  
 195                                      200                                      205  
 10 Ser Lys Ile Leu Val Leu Arg Ser Val Glu Tyr Ala Arg Ala Phe Asn  
 210                                      215                                      220  
 Val Pro Leu Arg Val Arg Ser Ser Tyr Ser Asn Asp Pro Gly Thr Leu  
 225                                      230                                      235                                      240  
 15 Ile Ala Gly Ser Met Glu Asp Ile Pro Val Glu Glu Ala Val Leu Thr  
 245                                      250                                      255  
 Gly Val Ala Thr Asp Lys Ser Glu Ala Lys Val Thr Val Leu Gly Ile  
 260                                      265                                      270  
 20 Ser Asp Lys Pro Gly Glu Ala Ala Lys Val Phe Arg Ala Leu Ala Asp  
 275                                      280                                      285  
 Ala Glu Ile Asn Ile Asp Met Val Leu Gln Asn Val Ser Ser Val Glu  
 290                                      295                                      300  
 Asp Gly Thr Thr Asp Ile Thr Phe Thr Cys Pro Arg Ala Asp Gly Arg  
 305                                      310                                      315                                      320  
 25 Arg Ala Met Glu Ile Leu Lys Lys Leu Gln Val Gln Gly Asn Trp Thr  
 325                                      330                                      335  
 Asn Val Leu Tyr Asp Asp Gln Val Gly Lys Val Ser Leu Val Gly Ala  
 340                                      345                                      350  
 30 Gly Met Lys Ser His Pro Gly Val Thr Ala Glu Phe Met Glu Ala Leu  
 355                                      360                                      365  
 Arg Asp Val Asn Val Asn Ile Glu Leu Ile Ser Thr Ser Glu Ile Arg  
 370                                      375                                      380  
 35 Ile Ser Val Leu Ile Arg Glu Asp Asp Leu Asp Ala Ala Ala Arg Ala  
 385                                      390                                      395                                      400  
 Leu His Glu Gln Phe Gln Leu Gly Gly Glu Asp Glu Ala Val Val Tyr  
 405                                      410                                      415  
 40 Ala Gly Thr Gly Arg  
 420

## (2) INFORMATION FOR SEQ ID NO:6:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1643 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

### (ii) MOLECULE TYPE: DNA (genomic)

### (iv) ANTI-SENSE: NO

### (vi) ORIGINAL SOURCE:

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(A) ORGANISM: Brevibacterium lactofermentum

(B) STRAIN: ATCC 13869

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 964..1482

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

	TCGOGAAGTA GCAOCTGTCA CTTTTGTCTC AAATATTAAA TCGAATATCA ATATAOGGTC	60
10	TGTTTATTGG AACGCATCCC AGTGGCTGAG ACGCATCCGC TAAAGCCCCA GGAACCCCTGT	120
	GCAGAAAGAA AACACTOCTC TGGCTAGGTA GACACAGTTT ATAAAGGTAG AGTTGAGCGG	180
	GTAAGTGTCA GCAOCTAGAT CGAAAGGTGC ACAAAGGTGG CCCTGGTGGT ACAGAAATAT	240
	GGGGTTTCTT CGCTTGAGAG TGGGGAACGC ATTAGAAAAG TGGCTGAAAG GATCGTTGCC	300
	ACCAAGAAGG CTGGAAATGA TGTCGTGGTT GTCTGCTCCG CAATGGGAGA CACCAOCCAT	360
15	GAAGTTCTAG AACTTGCAGC GGCAGTGAAT CCGGTTCCGC CAGCTCGTGA AATGGATATG	420
	CTOCTGACTG CTGGTGAGCG TATTTCTAAC GCTCTCGTGG CCATGGCTAT TGAGTCCCTT	480
	GGGCGAGAAG CTCAATCTTT CACTGGCTCT CAGGCTGGTG TGCTCAOCCAC CGAGCGOCCAC	540
	GGAAOCCAC GCATTGTTGA CGTCACACCG GGTCGTGTGC GTGAAGCACT CGATGAGGGC	600
20	AAGATCTGCA TTGTTGCTGG TTTTCAGGGT GTTAATAAAG AAACCCGCGA TGTCACCAAG	660
	TTGGGTGGTG GTGGTTCTGA CACCACTGCA GTTGGGTTGG CAGCTGCTTT GAAOCCGTAT	720
	GTGTGTGAGA TTTACTGGGA CGTTGAOCCG GTGTATACCG CTGACCCGCG CATCGTTCCCT	780
	AATGCACAGA AGCTGGAAAA GCTCAGCTTC GAAGAAATGC TGGAACTTGC TGCTGTTGGC	840
	TOCAAGATTT TGGTGTGGG CAGTGTGAA TACGCTGGTG CATTCAATGT GCCACTTCG	900
25	GTACGCTGGT CTTATAGTAA TGATCCCGGC ACTTTGATTG CCGGCTCTAT GGAGGATATT	960
	CCT GTG GAA GAA GCA GTC CTT ACC GGT GTC GCA ACC GAC AAG TOC GAA	1008
	Met Glu Glu Ala Val Leu Thr Gly Val Ala Thr Asp Lys Ser Glu	
	1 5 10 15	
30	GCC AAA GTA ACC GTT CTG GGT ATT TOC GAT AAG CCA GGC GAG GCT GGC	1056
	Ala Lys Val Thr Val Leu Gly Ile Ser Asp Lys Pro Gly Glu Ala Ala	
	20 25 30	
	AAG GTT TTC CGT GCG TTG GCT GAT GCA GAA ATC AAC ATT GAC ATG GTT	1104
	Lys Val Phe Arg Ala Leu Ala Asp Ala Glu Ile Asn Ile Asp Met Val	
35	35 40 45	
	CTG CAG AAC GTC TOC TCT GTG GAA GAC GGC ACC AOC GAC ATC AOC TTC	1152
	Leu Gln Asn Val Ser Ser Val Glu Asp Gly Thr Thr Asp Ile Thr Phe	
	50 55 60	
40	ACC TGC CCT CGC GCT GAC GGA CGC CGT GCG ATG GAG ATC TTG AAG AAG	1200
	Thr Cys Pro Arg Ala Asp Gly Arg Arg Ala Met Glu Ile Leu Lys Lys	
	65 70 75	
	CTT CAG GTT CAG GGC AAC TGG ACC AAT GTG CTT TAC GAC GAC CAG GTC	1248
	Leu Gln Val Gln Gly Asn Trp Thr Asn Val Leu Tyr Asp Asp Gln Val	
45	80 85 90 95	
	GGC AAA GTC TOC CTC GTG GGT GCT GGC ATG AAG TCT CAC CCA GGT GTT	1296
	Gly Lys Val Ser Leu Val Gly Ala Gly Met Lys Ser His Pro Gly Val	
	100 105 110	
50	ACC GCA GAG TTC ATG GAA GCT CTG CGC GAT GTC AAC GTG AAC ATC GAA	1344
	Thr Ala Glu Phe Met Glu Ala Leu Arg Asp Val Asn Val Asn Ile Glu	

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	115	120	125	
5	TTG ATT TOC AOC TCT GAG ATC OGC ATT TOC GTG CTG ATC OGT GAA GAT			1392
	Leu Ile Ser Thr Ser Glu Ile Arg Ile Ser Val Leu Ile Arg Glu Asp			
	130	135	140	
	GAT CTG GAT GCT GCT GCA OGT GCA TTG CAT GAG CAG TTC CAG CTG GGC			1440
	Asp Leu Asp Ala Ala Ala Arg Ala Leu His Glu Gln Phe Gln Leu Gly			
	145	150	155	
10	GGC GAA GAC GAA GCC GTC GTT TAT GCA GGC ACC GGA OGC TAAAGTTTAA			1490
	Gly Glu Asp Glu Ala Val Val Tyr Ala Gly Thr Gly Arg			
	160	165	170	
	AGGAGTAGTT TTACAATGAC CACCATCGCA GTTGTGGTG CAACCGGCCA GGTCGGCCAG			1550
	GTTATGCGCA CCTTTTGGGA AGAGCGCAAT TTCCAGCTG ACACTGTTTG TTTCTTTGCT			1610
15	TCCCCGGGTT CCGCAGGCOG TAAGATTGAA TTC			1643

## (2) INFORMATION FOR SEQ ID NO:7:

### (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 172 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

### (ii) MOLECULE TYPE: protein

### (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

25	Met Glu Glu Ala Val Leu Thr Gly Val Ala Thr Asp Lys Ser Glu Ala
	1 5 10 15
	Lys Val Thr Val Leu Gly Ile Ser Asp Lys Pro Gly Glu Ala Ala Lys
	20 25 30
30	Val Phe Arg Ala Leu Ala Asp Ala Glu Ile Asn Ile Asp Met Val Leu
	35 40 45
	Gln Asn Val Ser Ser Val Glu Asp Gly Thr Thr Asp Ile Thr Phe Thr
	50 55 60
35	Cys Pro Arg Ala Asp Gly Arg Arg Ala Met Glu Ile Leu Lys Lys Leu
	65 70 75 80
	Gln Val Gln Gly Asn Trp Thr Asn Val Leu Tyr Asp Asp Gln Val Gly
	85 90 95
	Lys Val Ser Leu Val Gly Ala Gly Met Lys Ser His Pro Gly Val Thr
	100 105 110
40	Ala Glu Phe Met Glu Ala Leu Arg Asp Val Asn Val Asn Ile Glu Leu
	115 120 125
	Ile Ser Thr Ser Glu Ile Arg Ile Ser Val Leu Ile Arg Glu Asp Asp
	130 135 140
45	Leu Asp Ala Ala Ala Arg Ala Leu His Glu Gln Phe Gln Leu Gly Gly
	145 150 155 160
	Glu Asp Glu Ala Val Val Tyr Ala Gly Thr Gly Arg
	165 170

## (2) INFORMATION FOR SEQ ID NO:8:



## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGATOOCCAA TCGATAOCTG GAA

23

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

## (iv) ANTI-SENSE: YES

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGTTTCATCG CCAAGTTTTT CTT

23

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2001 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Brevibacterium lactofermentum
- (B) STRAIN: ATCC 13869

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 730..1473

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGATOOCCAA	TOGATAOCTG	GAAOGACAAC	CTGATCAGGA	TATCCAATGC	CTTGAATATT	60
GACGTTGAGG	AAGGAATCAC	CAGOCATCTC	AACTGGAAGA	OCTGACGOCT	GCTGAATTGG	120
ATCAGTGGOC	CAATOGACOC	ACCAAOCAGG	TTGGCTATTA	OOGGCGATAT	CAAAAACAAC	180
TOGOGTGAAC	GTTTOGTGCT	CGGCAACGCG	GATGCCAGCG	ATCGACATAT	CGGAGTCACC	240
AACTTGAGCC	TGCTGCTTCT	GATOCATOGA	CGGGGAACCC	AACGGCGGCA	AAGCAGTGGG	300
GGAAGGGGAG	TTGGTGGACT	CTGAATCAGT	GGGCTCTGAA	GTGGTAGGCG	AOGGGGCAGC	360
ATCTGAAGGC	GTGOGAGTTG	TGGTGACCGG	GTTAGCGGTT	TCAGTTTCTG	TCACAACTGG	420

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	AGCAGGACTA GCAGAGGTTG TAGGCGTTGA GCGGCTTCCA TCACAAGCAC TTAAAAGTAA	480
	AGAGGCGGAA ACCACAAGCG CCAAGGAACT AOCTGCGGAA CGGGGCGTGA AGGGCAACTT	540
5	AAGTCTCATA TTTCAAACAT AGTTCCACCT GTGTGATTAA TCTCCAGAAC GGAACAAACT	600
	GATGAACAAT OGTTAACAAC ACAGAACAAA ACGGTCAGTT AGGTATGGAT ATCAGCACCT	660
	TCTGAATGGG TACGTCTAGA CTGGTGGGCG TTTGAAAAAC TCTTCGCCCC ACGAAAATGA	720
	AGGAGCATA ATG GGA ATC AAG GTT GGC GTT CTC GGA GCC AAA GGC CGT	768
	Met Gly Ile Lys Val Gly Val Leu Gly Ala Lys Gly Arg	
10	1 5 10	
	GTT GGT CAA ACT ATT GTG GCA GCA GTC AAT GAG TOC GAC GAT CTG GAG	816
	Val Gly Gln Thr Ile Val Ala Ala Val Asn Glu Ser Asp Asp Leu Glu	
	15 20 25	
15	CTT GTT GCA GAG ATC GGC GTC GAC GAT GAT TTG AGC CTT CTG GTA GAC	864
	Leu Val Ala Glu Ile Gly Val Asp Asp Asp Leu Ser Leu Leu Val Asp	
	30 35 40 45	
	AAC GGC GCT GAA GTT GTC GTT GAC TTC ACC ACT CCT AAC GCT GTG ATG	912
	Asn Gly Ala Glu Val Val Val Asp Phe Thr Thr Pro Asn Ala Val Met	
20	50 55 60	
	GGC AAC CTG GAG TTC TGC ATC AAC AAC GGC ATT TCT GCG GTT GTT GGA	960
	Gly Asn Leu Glu Phe Cys Ile Asn Asn Gly Ile Ser Ala Val Val Gly	
	65 70 75	
25	ACC ACG GGC TTC GAT GAT GCT CGT TTG GAG CAG GTT CGC GCC TGG CTT	1008
	Thr Thr Gly Phe Asp Asp Ala Arg Leu Glu Gln Val Arg Ala Trp Leu	
	80 85 90	
	GAA GGA AAA GAC AAT GTC GGT GTT CTG ATC GCA OCT AAC TTT GCT ATC	1056
	Glu Gly Lys Asp Asn Val Gly Val Leu Ile Ala Pro Asn Phe Ala Ile	
30	95 100 105	
	TCT GCG GTG TTG ACC ATG GTC TTT TOC AAG CAG GCT GCC CGC TTC TTC	1104
	Ser Ala Val Leu Thr Met Val Phe Ser Lys Gln Ala Ala Arg Phe Phe	
	110 115 120 125	
35	GAA TCA GCT GAA GTT ATT GAG CTG CAC CAC CCC AAC AAG CTG GAT GCA	1152
	Glu Ser Ala Glu Val Ile Glu Leu His His Pro Asn Lys Leu Asp Ala	
	130 135 140	
	OCT TCA GGC ACC GCG ATC CAC ACT GCT CAG GGC ATT GCT GCG GCA CGC	1200
	Pro Ser Gly Thr Ala Ile His Thr Ala Gln Gly Ile Ala Ala Ala Arg	
40	145 150 155	
	AAA GAA GCA GGC ATG GAC GCA CAG CCA GAT GCG ACC GAG CAG GCA CTT	1248
	Lys Glu Ala Gly Met Asp Ala Gln Pro Asp Ala Thr Glu Gln Ala Leu	
	160 165 170	
45	GAG GGT TOC CGT GGC GCA AGC GTA GAT GGA ATC CCA GTT CAC GCA GTC	1296
	Glu Gly Ser Arg Gly Ala Ser Val Asp Gly Ile Pro Val His Ala Val	
	175 180 185	
	CGC ATG TOC GGC ATG GTT GCT CAC GAG CAA GTT ATC TTT GGC ACC CAG	1344
	Arg Met Ser Gly Met Val Ala His Glu Gln Val Ile Phe Gly Thr Gln	
50	190 195 200 205	
	GGT CAG ACC TTG ACC ATC AAG CAG GAC TOC TAT GAT CGC AAC TCA TTT	1392

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Gly Gln Thr Leu Thr Ile Lys Gln Asp Ser Tyr Asp Arg Asn Ser Phe  
210 215 220  
5 GCA OCA GGT GTC TTG GTG GGT GTG CGC AAC ATT GCA CAG CAC OCA GGC 1440  
Ala Pro Gly Val Leu Val Gly Val Arg Asn Ile Ala Gln His Pro Gly  
225 230 235  
CTA GTC GTA GGA CTT GAG CAT TAC CTA GGC CTG TAAAGGCTCA TTTCAGCAGC 1493  
Leu Val Val Gly Leu Glu His Tyr Leu Gly Leu  
10 240 245  
GGGTGGAATT TTTTAAAAGG AGCGTTTAAA GGCTGTGGCC GAACAAGTTA AATTGAGCGT 1553  
GGAGTTGATA GCGTGCAGTT CTTTACTOC ACCCGCTGAT GTTGAGTGGT CAACTGATGT 1613  
TGAGGGGCGG GAAGCACTCG TCGAGTTTGC GGGTGGTGCC TGCTACGAAA CTTTGTGATAA 1673  
15 GCGAACCCT CGAACTGCTT CCAATGCTGC GTATCTGCGC CACATCATGG AAGTGGGGCA 1733  
CACTGCTTTG CTTGAGCATG CCAATGOCAC GATGTATATC CGAGGCATTT CTGGGTCCGC 1793  
GAOCCATGAA TTGGTCCGAC ACOGOCATTT TTCTTCTCT CAACTGTCTC AGCGTTTGGT 1853  
GCACAGCGGA GAATCGGAAG TAGTGGTGCC CACTCTCATC GATGAAGATC CGCAGTTGCG 1913  
20 TGAACTTTTC ATGCACGCCA TGGATGAGTC TCGGTTCGCT TTCAATGAGC TGCTTAATGC 1973  
GCTGGAAGAA AAACTTGGCG ATGAACCG 2001

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 248 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

30 Met Gly Ile Lys Val Gly Val Leu Gly Ala Lys Gly Arg Val Gly Gln  
1 5 10 15  
Thr Ile Val Ala Ala Val Asn Glu Ser Asp Asp Leu Glu Leu Val Ala  
20 25 30  
35 Glu Ile Gly Val Asp Asp Asp Leu Ser Leu Leu Val Asp Asn Gly Ala  
35 40 45  
Glu Val Val Val Asp Phe Thr Thr Pro Asn Ala Val Met Gly Asn Leu  
50 55 60  
40 Glu Phe Cys Ile Asn Asn Gly Ile Ser Ala Val Val Gly Thr Thr Gly  
65 70 75 80  
Phe Asp Asp Ala Arg Leu Glu Gln Val Arg Ala Trp Leu Glu Gly Lys  
85 90 95  
45 Asp Asn Val Gly Val Leu Ile Ala Pro Asn Phe Ala Ile Ser Ala Val  
100 105 110  
Leu Thr Met Val Phe Ser Lys Gln Ala Ala Arg Phe Phe Glu Ser Ala  
115 120 125  
Glu Val Ile Glu Leu His His Pro Asn Lys Leu Asp Ala Pro Ser Gly  
50 130 135 140  
Thr Ala Ile His Thr Ala Gln Gly Ile Ala Ala Ala Arg Lys Glu Ala  
145 150 155 160

Gly Met Asp Ala Gln Pro Asp Ala Thr Glu Gln Ala Leu Glu Gly Ser  
 165 170 175  
 Arg Gly Ala Ser Val Asp Gly Ile Pro Val His Ala Val Arg Met Ser  
 180 185 190  
 Gly Met Val Ala His Glu Gln Val Ile Phe Gly Thr Gln Gly Gln Thr  
 195 200 205  
 Leu Thr Ile Lys Gln Asp Ser Tyr Asp Arg Asn Ser Phe Ala Pro Gly  
 210 215 220  
 Val Leu Val Gly Val Arg Asn Ile Ala Gln His Pro Gly Leu Val Val  
 225 230 235 240  
 Gly Leu Glu His Tyr Leu Gly Leu  
 245

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTOGACGGAT CGCAAATGGC AAC

23

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

## (iv) ANTI-SENSE: YES

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGATOCCTTGA GCAOCTTGCG CAG

23

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1411 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iv) ANTI-SENSE: NO



## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Brevibacterium lactofermentum*

(B) STRAIN: ATCC 13869

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 311..1213

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

10	CTCTCGATAT CGAGAGAGAA GCAGCGCCAC GGTTTTTCGG TGATTTTGAG ATTGAAACTT	60
	TGGCAGACGG ATCGCAAATG GCAACAAGCC CGTATGTCAT GGACTTTTAA CGCAAAGCTC	120
	ACACCCACGA GCTAAAAATT CATATAGTTA AGACAACATT TTTGGCTGTA AAAGACAGCC	180
	GTAAAAACCT CTTGCTCATG TCAATTGTTT TTATCGGAAT GTGGCTTGGG CGATTGTTAT	240
15	GCAAAAGTTG TTAGGTTTTT TGCGGGGTTG TTAAACCCCC AAATGAGGGA AGAAGGTAAC	300
	CTTGAACTCT ATG AGC ACA GGT TTA ACA GCT AAG ACC GGA GTA GAG CAC	349
	Met Ser Thr Gly Leu Thr Ala Lys Thr Gly Val Glu His	
	1 5 10	
	TTC GGC ACC GTT GGA GTA GCA ATG GTT ACT CCA TTC ACG GAA TCC GGA	397
20	Phe Gly Thr Val Gly Val Ala Met Val Thr Pro Phe Thr Glu Ser Gly	
	15 20 25	
	GAC ATC GAT ATC GCT GCT GGC CGC GAA GTC GCG GCT TAT TTG GTT GAT	445
	Asp Ile Asp Ile Ala Ala Gly Arg Glu Val Ala Ala Tyr Leu Val Asp	
	30 35 40 45	
25	AAG GGC TTG GAT TCT TTG GTT CTC GCG GGC ACC ACT GGT GAA TCC CCA	493
	Lys Gly Leu Asp Ser Leu Val Leu Ala Gly Thr Thr Gly Glu Ser Pro	
	50 55 60	
	ACG ACA ACC GCC GCT GAA AAA CTA GAA CTG CTC AAG GCC GTT CGT GAG	541
30	Thr Thr Thr Ala Ala Glu Lys Leu Glu Leu Leu Lys Ala Val Arg Glu	
	65 70 75	
	GAA GTT GGG GAT CGG GCG AAC GTC ATC GCC GGT GTC GGA ACC AAC AAC	589
	Glu Val Gly Asp Arg Ala Asn Val Ile Ala Gly Val Gly Thr Asn Asn	
	80 85 90	
35	ACG CGG ACA TCT GTG GAA CTT GCG GAA GCT GCT GCT TCT GCT GGC GCA	637
	Thr Arg Thr Ser Val Glu Leu Ala Glu Ala Ala Ala Ser Ala Gly Ala	
	95 100 105	
	GAC GGC CTT TTA GTT GTA ACT CCT TAT TAC TCC AAG CCG AGC CAA GAG	685
40	Asp Gly Leu Leu Val Val Thr Pro Tyr Tyr Ser Lys Pro Ser Gln Glu	
	110 115 120 125	
	GGA TTG CTG GCG CAC TTC GGT GCA ATT GCT GCA GCA ACA GAG GTT CCA	733
	Gly Leu Leu Ala His Phe Gly Ala Ile Ala Ala Ala Thr Glu Val Pro	
	130 135 140	
45	ATT TGT CTC TAT GAC ATT OCT GGT CGG TCA GGT ATT CCA ATT GAG TCT	781
	Ile Cys Leu Tyr Asp Ile Pro Gly Arg Ser Gly Ile Pro Ile Glu Ser	
	145 150 155	
	GAT ACC ATG AGA CGC CTG AGT GAA TTA OCT ACG ATT TTG GCG GTC AAG	829
50	Asp Thr Met Arg Arg Leu Ser Glu Leu Pro Thr Ile Leu Ala Val Lys	
	160 165 170	

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5 GAC GGC AAG GGT GAC CTC GTT GCA GGC ACG TCA TTG ATC AAA GAA ACG 877  
 Asp Ala Lys Gly Asp Leu Val Ala Ala Thr Ser Leu Ile Lys Glu Thr  
 175 180 185  
 10 GGA CTT GGC TGG TAT TCA GGC GAT GAC CCA CTA AAC CTT GTT TGG CTT 925  
 Gly Leu Ala Trp Tyr Ser Gly Asp Asp Pro Leu Asn Leu Val Trp Leu  
 190 195 200 205  
 15 GCT TTG GGC GGA TCA GGT TTC ATT TCC GTA ATT GGA CAT GCA GCC CCC 973  
 Ala Leu Gly Gly Ser Gly Phe Ile Ser Val Ile Gly His Ala Ala Pro  
 210 215 220  
 20 ACA GCA TTA CGT GAG TTG TAC ACA AGC TTC GAG GAA GGC GAC CTC GTC 1021  
 Thr Ala Leu Arg Glu Leu Tyr Thr Ser Phe Glu Glu Gly Asp Leu Val  
 225 230 235  
 25 CGT GCG CCG GAA ATC AAC GCC AAA CTA TCA CCG CTG GTA GCT GCC CAA 1069  
 Arg Ala Arg Glu Ile Asn Ala Lys Leu Ser Pro Leu Val Ala Ala Gln  
 240 245 250  
 30 GGT CGC TTG GGT GGA GTC AGC TTG GCA AAA GCT GCT CTG CGT CTG CAG 1117  
 Gly Arg Leu Gly Gly Val Ser Leu Ala Lys Ala Ala Leu Arg Leu Gln  
 255 260 265  
 GGC ATC AAC GTA GGA GAT CCT CGA CTT CCA ATT ATG GCT CCA AAT GAG 1165  
 Gly Ile Asn Val Gly Asp Pro Arg Leu Pro Ile Met Ala Pro Asn Glu  
 270 275 280 285  
 35 CAG GAA CTT GAG GCT CTC CGA GAA GAC ATG AAA AAA GCT GGA GTT CTA 1213  
 Gln Glu Leu Glu Ala Leu Arg Glu Asp Met Lys Lys Ala Gly Val Leu  
 290 295 300  
 TAAATATGAA TGATTCCCGA AATCGCGGCC GGAAGGTTAC CCGCAAGGCG GCGCAACCAGA 1273  
 40 AGCTGGTCAG GAAAAOCATC TGGATAOCCC TGTCTTTTCAG GCAACCAGATG CTTCCTCTAA 1333  
 CCAGAGOGCT GTAAAAGCTG AGACCGOCCG AAACGACAAT CGGGATGCTG CGCAAGGTGC 1393  
 TCAAGGATOC CAACATTC 1411

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 301 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Ser Thr Gly Leu Thr Ala Lys Thr Gly Val Glu His Phe Gly Thr  
 1 5 10 15  
 Val Gly Val Ala Met Val Thr Pro Phe Thr Glu Ser Gly Asp Ile Asp  
 20 25 30  
 45 Ile Ala Ala Gly Arg Glu Val Ala Ala Tyr Leu Val Asp Lys Gly Leu  
 35 40 45  
 Asp Ser Leu Val Leu Ala Gly Thr Thr Gly Glu Ser Pro Thr Thr Thr  
 50 55 60  
 55 Ala Ala Glu Lys Leu Glu Leu Leu Lys Ala Val Arg Glu Glu Val Gly

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65                                      70                                      75                                      80  
 Asp Arg Ala Asn Val Ile Ala Gly Val Gly Thr Asn Asn Thr Arg Thr  
    85                                      90                                      95  
 5 Ser Val Glu Leu Ala Glu Ala Ala Ala Ser Ala Gly Ala Asp Gly Leu  
    100                                      105                                      110  
 Leu Val Val Thr Pro Tyr Tyr Ser Lys Pro Ser Gln Glu Gly Leu Leu  
    115                                      120                                      125  
 10 Ala His Phe Gly Ala Ile Ala Ala Ala Thr Glu Val Pro Ile Cys Leu  
    130                                      135                                      140  
 Tyr Asp Ile Pro Gly Arg Ser Gly Ile Pro Ile Glu Ser Asp Thr Met  
 145                                      150                                      155                                      160  
 15 Arg Arg Leu Ser Glu Leu Pro Thr Ile Leu Ala Val Lys Asp Ala Lys  
    165                                      170                                      175  
 Gly Asp Leu Val Ala Ala Thr Ser Leu Ile Lys Glu Thr Gly Leu Ala  
    180                                      185                                      190  
 Trp Tyr Ser Gly Asp Asp Pro Leu Asn Leu Val Trp Leu Ala Leu Gly  
 20                                      195                                      200                                      205  
 Gly Ser Gly Phe Ile Ser Val Ile Gly His Ala Ala Pro Thr Ala Leu  
    210                                      215                                      220  
 Arg Glu Leu Tyr Thr Ser Phe Glu Glu Gly Asp Leu Val Arg Ala Arg  
 25 225                                      230                                      235                                      240  
 Glu Ile Asn Ala Lys Leu Ser Pro Leu Val Ala Ala Gln Gly Arg Leu  
    245                                      250                                      255  
 Gly Gly Val Ser Leu Ala Lys Ala Ala Leu Arg Leu Gln Gly Ile Asn  
    260                                      265                                      270  
 30 Val Gly Asp Pro Arg Leu Pro Ile Met Ala Pro Asn Glu Gln Glu Leu  
    275                                      280                                      285  
 Glu Ala Leu Arg Glu Asp Met Lys Lys Ala Gly Val Leu  
    290                                      295                                      300

35 (2) INFORMATION FOR SEQ ID NO:16:  
       (i) SEQUENCE CHARACTERISTICS:  
           (A) LENGTH: 23 bases  
           (B) TYPE: nucleic acid  
 40        (C) STRANDEDNESS: single  
           (D) TOPOLOGY: linear  
       (ii) MOLECULE TYPE: other nucleic acid  
           (A) DESCRIPTION: /desc = "synthetic DNA"  
       (iv) ANTI-SENSE: NO  
 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:  
 GTGGAGCCGA CCAATTCGCG AGG

23

50 (2) INFORMATION FOR SEQ ID NO:17:  
       (i) SEQUENCE CHARACTERISTICS:  
           (A) LENGTH: 23 bases

55

(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "synthetic DNA"

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

OCAAAACCGC CCTCCACGGC GAA

23

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3579 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Brevibacterium lactofermentum

(B) STRAIN: ATCC 13869

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 533..2182

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 2188..3522

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTGGAGCOGA	OCATTCCGCG	AGGCTGCACT	GCAACGAGGT	CGTAGTTTGT	GTACATGGCT	60
TCTGGCCAGT	TCATGGATTG	GCTGCOGAAG	AAGCTATAGG	CATCGCACCA	GGGCCACCGA	120
GTTACCGAAG	ATGGTGCCGT	GCTTTTCGCG	TTGGGCAGGG	ACCTTGACAA	AGCCACGCT	180
GATATCGOCA	AGTGAGGGAT	CAGAATAGTG	CATGGGCACG	TCGATGCTGC	CACATTGAGC	240
GGAGGCAATA	TCTACCTGAG	GTGGGCATTC	TTCCACGCGG	ATGTTTCTCT	GCGCTGCTGC	300
AGTGGGCATT	GATAACAAA	AGGGGCTAAG	CGCAGTCGAG	GCGGCAAGAA	CTGCTACTAC	360
OCTTTTATT	GTCGAACGGG	GCATTACGGC	TOCAAGGACG	TTTGTTTCT	GGGTCAGTTA	420
COCCAAAAG	CATATACAGA	GACCAATGAT	TTTTCATTAA	AAAGGCAGGG	ATTTGTTATA	480
AGTATGGGTC	GTATTCTGTG	CGACGGGTGT	ACCTCGGCTA	GAATTTCTCC	CC ATG	535

Met

1

ACA	CCA	GCT	GAT	CTC	GCA	ACA	TTG	ATT	AAA	GAG	ACC	GCG	GTA	GAG	GTT	583
Thr	Pro	Ala	Asp	Leu	Ala	Thr	Leu	Ile	Lys	Glu	Thr	Ala	Val	Glu	Val	

5

10

15

TTG	AOC	TOC	CGC	GAG	CTC	GAT	ACT	TCT	GTT	CTT	COG	GAG	CAG	GTA	GTT	631
Leu	Thr	Ser	Arg	Glu	Leu	Asp	Thr	Ser	Val	Leu	Pro	Glu	Gln	Val	Val	

20

25

30

GTG	GAG	CGT	COG	OGT	AAC	CCA	GAG	CAC	GGC	GAT	TAC	GCC	ACC	AAC	ATT	679
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----



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	Val	Glu	Arg	Pro	Arg	Asn	Pro	Glu	His	Gly	Asp	Tyr	Ala	Thr	Asn	Ile	
	35						40					45					
5	GCA	TTG	CAG	GTG	GCT	AAA	AAG	GTC	GGT	CAG	AAC	OCT	OGG	GAT	TTG	GCT	727
	Ala	Leu	Gln	Val	Ala	Lys	Lys	Val	Gly	Gln	Asn	Pro	Arg	Asp	Leu	Ala	
	50					55					60				65		
	ACC	TGG	CTG	GCA	GAG	GCA	TTG	GCT	GCA	GAT	GAC	GOC	ATT	GAT	TCT	GCT	775
	Thr	Trp	Leu	Ala	Glu	Ala	Leu	Ala	Ala	Asp	Asp	Ala	Ile	Asp	Ser	Ala	
10					70				75					80			
	GAA	ATT	GCT	GGC	CCA	GGC	TTT	TTG	AAC	ATT	CGC	CTT	GCT	GCA	GCA	GCA	823
	Glu	Ile	Ala	Gly	Pro	Gly	Phe	Leu	Asn	Ile	Arg	Leu	Ala	Ala	Ala	Ala	
				85				90					95				
15	CAG	GGT	GAA	ATT	GTG	GCC	AAG	ATT	CTG	GCA	CAG	GGC	GAG	ACT	TTC	GGA	871
	Gln	Gly	Glu	Ile	Val	Ala	Lys	Ile	Leu	Ala	Gln	Gly	Glu	Thr	Phe	Gly	
				100				105					110				
	AAC	TOC	GAT	CAC	CTT	TOC	CAC	TTG	GAC	GTG	AAC	CTC	GAG	TTC	GTT	TCT	919
	Asn	Ser	Asp	His	Leu	Ser	His	Leu	Asp	Val	Asn	Leu	Glu	Phe	Val	Ser	
20				115				120					125				
	GCA	AAC	CCA	ACC	GGA	CCT	ATT	CAC	CTT	GGC	GGA	ACC	CGC	TGG	GCT	GCC	967
	Ala	Asn	Pro	Thr	Gly	Pro	Ile	His	Leu	Gly	Gly	Thr	Arg	Trp	Ala	Ala	
	130					135					140				145		
25	GTG	GGT	GAC	TCT	TTG	GGT	CGT	GTG	CTG	GAG	GCT	TOC	GGC	GCG	AAA	GTG	1015
	Val	Gly	Asp	Ser	Leu	Gly	Arg	Val	Leu	Glu	Ala	Ser	Gly	Ala	Lys	Val	
					150					155					160		
	ACC	CGC	GAA	TAC	TAC	TTC	AAC	GAT	CAC	GGT	CGC	CAG	ATC	GAT	CGT	TTC	1063
	Thr	Arg	Glu	Tyr	Tyr	Phe	Asn	Asp	His	Gly	Arg	Gln	Ile	Asp	Arg	Phe	
30				165				170					175				
	GCT	TTG	TOC	CTT	CTT	GCA	GCG	GCG	AAG	GGC	GAG	CCA	ACG	CCA	GAA	GAC	1111
	Ala	Leu	Ser	Leu	Leu	Ala	Ala	Ala	Lys	Gly	Glu	Pro	Thr	Pro	Glu	Asp	
				180				185					190				
35	GGT	TAT	GGC	GGC	GAA	TAC	ATT	AAG	GAA	ATT	GCG	GAG	GCA	ATC	GTC	GAA	1159
	Gly	Tyr	Gly	Gly	Glu	Tyr	Ile	Lys	Glu	Ile	Ala	Glu	Ala	Ile	Val	Glu	
				195			200				205						
	AAG	CAT	CCT	GAA	GCG	TTG	GCT	TTG	GAG	CCT	GCC	GCA	ACC	CAG	GAG	CTT	1207
	Lys	His	Pro	Glu	Ala	Leu	Ala	Leu	Glu	Pro	Ala	Ala	Thr	Gln	Glu	Leu	
40					210			215				220			225		
	TTC	CGC	GCT	GAA	GGC	GTG	GAG	ATG	ATG	TTC	GAG	CAC	ATC	AAA	TCT	TOC	1255
	Phe	Arg	Ala	Glu	Gly	Val	Glu	Met	Met	Phe	Glu	His	Ile	Lys	Ser	Ser	
					230					235				240			
45	CTG	CAT	GAG	TTC	GGC	ACC	GAT	TTC	GAT	GTC	TAC	TAC	CAC	GAG	AAC	TOC	1303
	Leu	His	Glu	Phe	Gly	Thr	Asp	Phe	Asp	Val	Tyr	Tyr	His	Glu	Asn	Ser	
				245						250				255			
	CTG	TTC	GAG	TOC	GGT	GCG	GTG	GAC	AAG	GCC	GTG	CAG	GTG	CTG	AAG	GAC	1351
	Leu	Phe	Glu	Ser	Gly	Ala	Val	Asp	Lys	Ala	Val	Gln	Val	Leu	Lys	Asp	
50				260				265					270				
	AAC	GGC	AAC	CTG	TAC	GAA	AAC	GAG	GCC	GCT	TGG	TGG	CTG	CGT	TOC	ACC	1399

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	Asn	Gly	Asn	Leu	Tyr	Glu	Asn	Glu	Gly	Ala	Trp	Trp	Leu	Arg	Ser	Thr	
	275						280				285						
5	GAA	TTC	GGC	GAT	GAC	AAA	GAC	OGC	GTG	GTG	ATC	AAG	TCT	GAC	GGC	GAC	1447
	Glu	Phe	Gly	Asp	Asp	Lys	Asp	Arg	Val	Val	Ile	Lys	Ser	Asp	Gly	Asp	
	290					295					300					305	
	GCA	GCC	TAC	ATC	GCT	GGC	GAT	ATC	GCG	TAC	GTG	GCT	GAT	AAG	TTC	TOC	1495
	Ala	Ala	Tyr	Ile	Ala	Gly	Asp	Ile	Ala	Tyr	Val	Ala	Asp	Lys	Phe	Ser	
10					310					315					320		
	OGC	GGA	CAC	AAC	CTA	AAC	ATC	TAC	ATG	TTG	GGT	GCT	GAC	CAC	CAT	GGT	1543
	Arg	Gly	His	Asn	Leu	Asn	Ile	Tyr	Met	Leu	Gly	Ala	Asp	His	His	Gly	
				325				330					335				
15	TAC	ATC	GCG	OGC	CTG	AAG	GCA	GCG	GCG	GCG	GCA	CTT	GGC	TAC	AAG	CCA	1591
	Tyr	Ile	Ala	Arg	Leu	Lys	Ala	Ala	Ala	Ala	Ala	Leu	Gly	Tyr	Lys	Pro	
			340				345					350					
	GAA	GGC	GTT	GAA	GTC	CTG	ATT	GGC	CAG	ATG	GTG	AAC	CTG	CTT	OGC	GAC	1639
	Glu	Gly	Val	Glu	Val	Leu	Ile	Gly	Gln	Met	Val	Asn	Leu	Leu	Arg	Asp	
20		355				360					365						
	GGC	AAG	GCA	GTG	CGT	ATG	TOC	AAG	OGT	GCA	GGC	AOC	GTG	GTC	AOC	CTA	1687
	Gly	Lys	Ala	Val	Arg	Met	Ser	Lys	Arg	Ala	Gly	Thr	Val	Val	Thr	Leu	
	370				375				380						385		
25	GAT	GAC	CTC	GTT	GAA	GCA	ATC	GGC	ATC	GAT	GCG	GCG	CGT	TAC	TOC	CTG	1735
	Asp	Asp	Leu	Val	Glu	Ala	Ile	Gly	Ile	Asp	Ala	Ala	Arg	Tyr	Ser	Leu	
				390				395					400				
	ATC	OGT	TOC	TOC	GTG	GAT	TCT	TOC	CTG	GAT	ATC	GAT	CTC	GGC	CTG	TGG	1783
	Ile	Arg	Ser	Ser	Val	Asp	Ser	Ser	Leu	Asp	Ile	Asp	Leu	Gly	Leu	Trp	
30				405				410					415				
	GAA	TOC	CAG	TOC	TOC	GAC	AAC	OCT	GTG	TAC	TAC	GTG	CAG	TAC	GGA	CAC	1831
	Glu	Ser	Gln	Ser	Ser	Asp	Asn	Pro	Val	Tyr	Tyr	Val	Gln	Tyr	Gly	His	
		420					425					430					
35	GCT	OGT	CTG	TGC	TOC	ATC	GCG	OGC	AAG	GCA	GAG	AOC	TTG	GGT	GTC	AOC	1879
	Ala	Arg	Leu	Cys	Ser	Ile	Ala	Arg	Lys	Ala	Glu	Thr	Leu	Gly	Val	Thr	
		435				440					445						
	GAG	GAA	GGC	GCA	GAC	CTA	TCT	CTA	CTG	AOC	CAC	GAC	OGC	GAA	GGC	GAT	1927
	Glu	Glu	Gly	Ala	Asp	Leu	Ser	Leu	Leu	Thr	His	Asp	Arg	Glu	Gly	Asp	
40		450				455				460					465		
	CTC	ATC	OGC	ACA	CTC	GGA	GAG	TTC	CCA	GCA	GTG	GTG	AAG	GCT	GCC	GCT	1975
	Leu	Ile	Arg	Thr	Leu	Gly	Glu	Phe	Pro	Ala	Val	Val	Lys	Ala	Ala	Ala	
				470				475					480				
45	GAC	CTA	OGT	GAA	CCA	CAC	OGC	ATT	GOC	OGC	TAT	GCT	GAG	GAA	TTA	GCT	2023
	Asp	Leu	Arg	Glu	Pro	His	Arg	Ile	Ala	Arg	Tyr	Ala	Glu	Glu	Leu	Ala	
			485					490				495					
	GGA	ACT	TTC	CAC	OGC	TTC	TAC	GAT	TOC	TGC	CAC	ATC	CTT	CCA	AAG	GTT	2071
	Gly	Thr	Phe	His	Arg	Phe	Tyr	Asp	Ser	Cys	His	Ile	Leu	Pro	Lys	Val	
50			500					505				510					
	GAT	GAG	GAT	ACG	GCA	CCA	ATC	CAC	ACA	GCA	OGT	CTG	GCA	CTT	GCA	GCA	2119

55

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	Asp Glu Asp Thr Ala Pro Ile His Thr Ala Arg Leu Ala Leu Ala Ala	
	515 520 525	
5	GCA ACC OGC CAG ACC CTC GCT AAC GCC CTG CAC CTG GTT GGC GTT TOC	2167
	Ala Thr Arg Gln Thr Leu Ala Asn Ala Leu His Leu Val Gly Val Ser	
	530 535 540 545	
	GCA CCG GAG AAG ATG TAACA ATG GCT ACA GTT GAA AAT TTC AAT GAA	2214
	Ala Pro Glu Lys Met Met Ala Thr Val Glu Asn Phe Asn Glu	
10	550 1 5	
	CTT CCC GCA CAC GTA TGG CCA CGC AAT GCC GTG CGC CAA GAA GAC GGC	2262
	Leu Pro Ala His Val Trp Pro Arg Asn Ala Val Arg Gln Glu Asp Gly	
	10 15 20 25	
15	GTT GTC ACC GTC GCT GGT GTG OCT CTG OCT GAC CTC GCT GAA GAA TAC	2310
	Val Val Thr Val Ala Gly Val Pro Leu Pro Asp Leu Ala Glu Glu Tyr	
	30 35 40	
	GGA ACC CCA CTG TTC GTA GTC GAC GAG GAC GAT TTC CGT TOC CGC TGT	2358
	Gly Thr Pro Leu Phe Val Val Asp Glu Asp Asp Phe Arg Ser Arg Cys	
20	45 50 55	
	OGC GAC ATG GCT ACC GCA TTC GGT GGA CCA GGC AAT GTG CAC TAC GCA	2406
	Arg Asp Met Ala Thr Ala Phe Gly Gly Pro Gly Asn Val His Tyr Ala	
	60 65 70	
25	TCT AAA GCG TTC CTG ACC AAG ACC ATT GCA CGT TGG GTT GAT GAA GAG	2454
	Ser Lys Ala Phe Leu Thr Lys Thr Ile Ala Arg Trp Val Asp Glu Glu	
	75 80 85	
	GGG CTG GCA CTG GAC ATT GCA TOC ATC AAC GAA CTG GGC ATT GOC CTG	2502
	Gly Leu Ala Leu Asp Ile Ala Ser Ile Asn Glu Leu Gly Ile Ala Leu	
30	90 95 100 105	
	GOC GCT GGT TTC CCC GCC AGC CGT ATC ACC GCG CAC GGC AAC AAC AAA	2550
	Ala Ala Gly Phe Pro Ala Ser Arg Ile Thr Ala His Gly Asn Asn Lys	
	110 115 120	
35	GGC GTA GAG TTC CTG CGC GCG TTG GTT CAA AAC GGT GTG GGA CAC GTG	2598
	Gly Val Glu Phe Leu Arg Ala Leu Val Gln Asn Gly Val Gly His Val	
	125 130 135	
	GTG CTG GAC TOC GCA CAG GAA CTA GAA CTG TTG GAT TAC GTT GOC GCT	2646
	Val Leu Asp Ser Ala Gln Glu Leu Glu Leu Leu Asp Tyr Val Ala Ala	
40	140 145 150	
	GGT GAA GGC AAG ATT CAG GAC GTG TTG ATC CGC GTA AAG CCA GGC ATC	2694
	Gly Glu Gly Lys Ile Gln Asp Val Leu Ile Arg Val Lys Pro Gly Ile	
	155 160 165	
45	GAA GCA CAC ACC CAC GAG TTC ATC GOC ACT AGC CAC GAA GAC CAG AAG	2742
	Glu Ala His Thr His Glu Phe Ile Ala Thr Ser His Glu Asp Gln Lys	
	170 175 180 185	
	TTC GGA TTC TOC CTG GCA TOC GGT TOC GCA TTC GAA GCA GCA AAA GOC	2790
	Phe Gly Phe Ser Leu Ala Ser Gly Ser Ala Phe Glu Ala Ala Lys Ala	
50	190 195 200	
	GCC AAC AAC GCA GAA AAC CTG AAC CTG GTT GGC CTG CAC TGC CAC GTT	2838

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	Ala	Asn	Asn	Ala	Glu	Asn	Leu	Asn	Leu	Val	Gly	Leu	His	Cys	His	Val	
				205					210					215			
5	GGT	TOC	CAG	GTG	TTC	GAC	GOC	GAA	GGC	TTC	AAG	CTG	GCA	GCA	GAA	OGC	2886
	Gly	Ser	Gln	Val	Phe	Asp	Ala	Glu	Gly	Phe	Lys	Leu	Ala	Ala	Glu	Arg	
			220					225				230					
	GTG	TTG	GGC	CTG	TAC	TCA	CAG	ATC	CAC	AGC	GAA	CTG	GGC	GTT	GOC	CTT	2934
	Val	Leu	Gly	Leu	Tyr	Ser	Gln	Ile	His	Ser	Glu	Leu	Gly	Val	Ala	Leu	
10		235					240			245							
	OCT	GAA	CTG	GAT	CTC	GGT	GGC	GGA	TAC	GGC	ATT	GOC	TAT	AOC	GCA	GCT	2982
	Pro	Glu	Leu	Asp	Leu	Gly	Gly	Gly	Tyr	Gly	Ile	Ala	Tyr	Thr	Ala	Ala	
	250				255				260						265		
15	GAA	GAA	CCA	CTC	AAC	GTC	GCA	GAA	GTT	GCC	TOC	GAC	CTG	CTC	AOC	GCA	3030
	Glu	Glu	Pro	Leu	Asn	Val	Ala	Glu	Val	Ala	Ser	Asp	Leu	Leu	Thr	Ala	
				270				275					280				
	GTC	GGA	AAA	ATG	GCA	GCG	GAA	CTA	GGC	ATC	GAC	GCA	CCA	AOC	GTG	CTT	3078
	Val	Gly	Lys	Met	Ala	Ala	Glu	Leu	Gly	Ile	Asp	Ala	Pro	Thr	Val	Leu	
20			285					290				295					
	GTT	GAG	CCC	GGC	CGC	GCT	ATC	GCA	GGC	CCC	TOC	AOC	GTG	AOC	ATC	TAC	3126
	Val	Glu	Pro	Gly	Arg	Ala	Ile	Ala	Gly	Pro	Ser	Thr	Val	Thr	Ile	Tyr	
		300					305			310							
25	GAA	GTC	GGC	ACC	ACC	AAA	GAC	GTC	CAC	GTA	GAC	GAC	GAC	AAA	ACC	CGC	3174
	Glu	Val	Gly	Thr	Thr	Lys	Asp	Val	His	Val	Asp	Asp	Asp	Lys	Thr	Arg	
		315				320			325								
	CGT	TAC	ATC	GOC	GTG	GAC	GGA	GGC	ATG	TOC	GAC	AAC	ATC	OGC	CCA	GCA	3222
	Arg	Tyr	Ile	Ala	Val	Asp	Gly	Gly	Met	Ser	Asp	Asn	Ile	Arg	Pro	Ala	
30	330				335				340				345				
	CTC	TAC	GGC	TOC	GAA	TAC	GAC	GOC	OGC	GTA	GTA	TOC	OGC	TTC	GOC	GAA	3270
	Leu	Tyr	Gly	Ser	Glu	Tyr	Asp	Ala	Arg	Val	Val	Ser	Arg	Phe	Ala	Glu	
				350				355					360				
35	GGA	GAC	CCA	GTA	AGC	AOC	CGC	ATC	GTG	GGC	TOC	CAC	TGC	GAA	TOC	GGC	3318
	Gly	Asp	Pro	Val	Ser	Thr	Arg	Ile	Val	Gly	Ser	His	Cys	Glu	Ser	Gly	
			365					370				375					
	GAT	ATC	CTG	ATC	AAC	GAT	GAA	ATC	TAC	CCA	TCT	GAC	ATC	ACC	AGC	GGC	3366
	Asp	Ile	Leu	Ile	Asn	Asp	Glu	Ile	Tyr	Pro	Ser	Asp	Ile	Thr	Ser	Gly	
40			380				385			390							
	GAC	TTC	CTT	GCA	CTC	GCA	GOC	ACC	GGC	GCA	TAC	TGC	TAC	GOC	ATG	AGC	3414
	Asp	Phe	Leu	Ala	Leu	Ala	Ala	Thr	Gly	Ala	Tyr	Cys	Tyr	Ala	Met	Ser	
		395				400			405								
45	TOC	OGC	TAC	AAC	GOC	TTC	ACA	CGG	CCC	GOC	GTG	GTG	TOC	GTG	OGC	GCT	3462
	Ser	Arg	Tyr	Asn	Ala	Phe	Thr	Arg	Pro	Ala	Val	Val	Ser	Val	Arg	Ala	
	410			415				420				425					
	GGC	AGC	TOC	CGC	CTC	ATG	CTG	CGC	OGC	GAA	ACG	CTC	GAC	GAC	ATC	CTC	3510
	Gly	Ser	Ser	Arg	Leu	Met	Leu	Arg	Arg	Glu	Thr	Leu	Asp	Asp	Ile	Leu	
50				430				435				440					
	TCA	CTA	GAG	GCA	TAACGCTTTT	CGACGCTGA	CCCCGCOCTT	CAOCTTCGOC									3562



Ser Leu Glu Ala  
445  
GTGGAGGGGCG GTTTTGG

3579

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 550 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Thr Pro Ala Asp Leu Ala Thr Leu Ile Lys Glu Thr Ala Val Glu  
1 5 10 15  
Val Leu Thr Ser Arg Glu Leu Asp Thr Ser Val Leu Pro Glu Gln Val  
20 25 30  
Val Val Glu Arg Pro Arg Asn Pro Glu His Gly Asp Tyr Ala Thr Asn  
35 40 45  
Ile Ala Leu Gln Val Ala Lys Lys Val Gly Gln Asn Pro Arg Asp Leu  
50 55 60  
Ala Thr Trp Leu Ala Glu Ala Leu Ala Ala Asp Asp Ala Ile Asp Ser  
65 70 75 80  
Ala Glu Ile Ala Gly Pro Gly Phe Leu Asn Ile Arg Leu Ala Ala Ala  
85 90 95  
Ala Gln Gly Glu Ile Val Ala Lys Ile Leu Ala Gln Gly Glu Thr Phe  
100 105 110  
Gly Asn Ser Asp His Leu Ser His Leu Asp Val Asn Leu Glu Phe Val  
115 120 125  
Ser Ala Asn Pro Thr Gly Pro Ile His Leu Gly Gly Thr Arg Trp Ala  
130 135 140  
Ala Val Gly Asp Ser Leu Gly Arg Val Leu Glu Ala Ser Gly Ala Lys  
145 150 155 160  
Val Thr Arg Glu Tyr Tyr Phe Asn Asp His Gly Arg Gln Ile Asp Arg  
165 170 175  
Phe Ala Leu Ser Leu Leu Ala Ala Ala Lys Gly Glu Pro Thr Pro Glu  
180 185 190  
Asp Gly Tyr Gly Gly Glu Tyr Ile Lys Glu Ile Ala Glu Ala Ile Val  
195 200 205  
Glu Lys His Pro Glu Ala Leu Ala Leu Glu Pro Ala Ala Thr Gln Glu  
210 215 220  
Leu Phe Arg Ala Glu Gly Val Glu Met Met Phe Glu His Ile Lys Ser  
225 230 235 240  
Ser Leu His Glu Phe Gly Thr Asp Phe Asp Val Tyr Tyr His Glu Asn  
245 250 255  
Ser Leu Phe Glu Ser Gly Ala Val Asp Lys Ala Val Gln Val Leu Lys  
260 265 270

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Asp Asn Gly Asn Leu Tyr Glu Asn Glu Gly Ala Trp Trp Leu Arg Ser  
275 280 285  
5 Thr Glu Phe Gly Asp Asp Lys Asp Arg Val Val Ile Lys Ser Asp Gly  
290 295 300  
Asp Ala Ala Tyr Ile Ala Gly Asp Ile Ala Tyr Val Ala Asp Lys Phe  
305 310 315 320  
10 Ser Arg Gly His Asn Leu Asn Ile Tyr Met Leu Gly Ala Asp His His  
325 330 335  
Gly Tyr Ile Ala Arg Leu Lys Ala Ala Ala Ala Leu Gly Tyr Lys  
340 345 350  
15 Pro Glu Gly Val Glu Val Leu Ile Gly Gln Met Val Asn Leu Leu Arg  
355 360 365  
Asp Gly Lys Ala Val Arg Met Ser Lys Arg Ala Gly Thr Val Val Thr  
370 375 380  
20 Leu Asp Asp Leu Val Glu Ala Ile Gly Ile Asp Ala Ala Arg Tyr Ser  
385 390 395 400  
Leu Ile Arg Ser Ser Val Asp Ser Ser Leu Asp Ile Asp Leu Gly Leu  
405 410 415  
Trp Glu Ser Gln Ser Ser Asp Asn Pro Val Tyr Tyr Val Gln Tyr Gly  
420 425 430  
25 His Ala Arg Leu Cys Ser Ile Ala Arg Lys Ala Glu Thr Leu Gly Val  
435 440 445  
Thr Glu Glu Gly Ala Asp Leu Ser Leu Leu Thr His Asp Arg Glu Gly  
450 455 460  
30 Asp Leu Ile Arg Thr Leu Gly Glu Phe Pro Ala Val Val Lys Ala Ala  
465 470 475 480  
Ala Asp Leu Arg Glu Pro His Arg Ile Ala Arg Tyr Ala Glu Glu Leu  
485 490 495  
35 Ala Gly Thr Phe His Arg Phe Tyr Asp Ser Cys His Ile Leu Pro Lys  
500 505 510  
Val Asp Glu Asp Thr Ala Pro Ile His Thr Ala Arg Leu Ala Leu Ala  
515 520 525  
40 Ala Ala Thr Arg Gln Thr Leu Ala Asn Ala Leu His Leu Val Gly Val  
530 535 540  
Ser Ala Pro Glu Lys Met  
545 550

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 445 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Ala Thr Val Glu Asn Phe Asn Glu Leu Pro Ala His Val Trp Pro

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	1			5					10					15		
	Arg	Asn	Ala	Val	Arg	Gln	Glu	Asp	Gly	Val	Val	Thr	Val	Ala	Gly	Val
				20					25					30		
5	Pro	Leu	Pro	Asp	Leu	Ala	Glu	Glu	Tyr	Gly	Thr	Pro	Leu	Phe	Val	Val
			35					40					45			
	Asp	Glu	Asp	Asp	Phe	Arg	Ser	Arg	Cys	Arg	Asp	Met	Ala	Thr	Ala	Phe
		50					55					60				
10	Gly	Gly	Pro	Gly	Asn	Val	His	Tyr	Ala	Ser	Lys	Ala	Phe	Leu	Thr	Lys
	65					70					75					80
	Thr	Ile	Ala	Arg	Trp	Val	Asp	Glu	Glu	Gly	Leu	Ala	Leu	Asp	Ile	Ala
					85					90					95	
15	Ser	Ile	Asn	Glu	Leu	Gly	Ile	Ala	Leu	Ala	Ala	Gly	Phe	Pro	Ala	Ser
			100						105				110			
	Arg	Ile	Thr	Ala	His	Gly	Asn	Asn	Lys	Gly	Val	Glu	Phe	Leu	Arg	Ala
			115					120					125			
20	Leu	Val	Gln	Asn	Gly	Val	Gly	His	Val	Val	Leu	Asp	Ser	Ala	Gln	Glu
		130					135					140				
	Leu	Glu	Leu	Leu	Asp	Tyr	Val	Ala	Ala	Gly	Glu	Gly	Lys	Ile	Gln	Asp
	145					150					155					160
	Val	Leu	Ile	Arg	Val	Lys	Pro	Gly	Ile	Glu	Ala	His	Thr	His	Glu	Phe
					165					170					175	
25	Ile	Ala	Thr	Ser	His	Glu	Asp	Gln	Lys	Phe	Gly	Phe	Ser	Leu	Ala	Ser
				180					185					190		
	Gly	Ser	Ala	Phe	Glu	Ala	Ala	Lys	Ala	Ala	Asn	Asn	Ala	Glu	Asn	Leu
			195					200					205			
30	Asn	Leu	Val	Gly	Leu	His	Cys	His	Val	Gly	Ser	Gln	Val	Phe	Asp	Ala
		210					215					220				
	Glu	Gly	Phe	Lys	Leu	Ala	Ala	Glu	Arg	Val	Leu	Gly	Leu	Tyr	Ser	Gln
	225					230					235					240
35	Ile	His	Ser	Glu	Leu	Gly	Val	Ala	Leu	Pro	Glu	Leu	Asp	Leu	Gly	Gly
					245					250					255	
	Gly	Tyr	Gly	Ile	Ala	Tyr	Thr	Ala	Ala	Glu	Glu	Pro	Leu	Asn	Val	Ala
				260					265					270		
40	Glu	Val	Ala	Ser	Asp	Leu	Leu	Thr	Ala	Val	Gly	Lys	Met	Ala	Ala	Glu
			275					280					285			
	Leu	Gly	Ile	Asp	Ala	Pro	Thr	Val	Leu	Val	Glu	Pro	Gly	Arg	Ala	Ile
		290					295					300				
45	Ala	Gly	Pro	Ser	Thr	Val	Thr	Ile	Tyr	Glu	Val	Gly	Thr	Thr	Lys	Asp
	305					310					315					320
	Val	His	Val	Asp	Asp	Asp	Lys	Thr	Arg	Arg	Tyr	Ile	Ala	Val	Asp	Gly
					325					330					335	
	Gly	Met	Ser	Asp	Asn											

Ile Val Gly Ser His Cys Glu Ser Gly Asp Ile Leu Ile Asn Asp Glu  
 370 375 380  
 Ile Tyr Pro Ser Asp Ile Thr Ser Gly Asp Phe Leu Ala Leu Ala Ala  
 5 385 390 395 400  
 Thr Gly Ala Tyr Cys Tyr Ala Met Ser Ser Arg Tyr Asn Ala Phe Thr  
 405 410 415  
 Arg Pro Ala Val Val Ser Val Arg Ala Gly Ser Ser Arg Leu Met Leu  
 10 420 425 430  
 Arg Arg Glu Thr Leu Asp Asp Ile Leu Ser Leu Glu Ala  
 435 440 445

## (2) INFORMATION FOR SEQ ID NO:21:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

## (iv) ANTI-SENSE: NO

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CATCTAAGTA TGCATCTCGG

20

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "synthetic DNA"

## (iv) ANTI-SENSE: YES

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGCCOCTCGA GCTAAATTAG

20

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1034 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (iv) ANTI-SENSE: NO

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Brevibacterium lactofermentum



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(B) STRAIN: ATCC 13869

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 61..1020

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

	ATGCATCTCG GTAAGCTCGA CCAGGACAGT GCAACACAA TTTTGGAGGA TTACAAGAAC	60
	ATG ACC AAC ATC OGC GTA GCT ATC GTG GGC TAC GGA AAC CTG GGA CGC	108
5	Met Thr Asn Ile Arg Val Ala Ile Val Gly Tyr Gly Asn Leu Gly Arg	
	1 5 10 15	
	AGC GTC GAA AAG CTT ATT GOC AAG CAG CCC GAC ATG GAC CTT GTA GGA	156
	Ser Val Glu Lys Leu Ile Ala Lys Gln Pro Asp Met Asp Leu Val Gly	
	20 25 30	
15	ATC TTC TOG CGC CGG GCC ACC CTC GAC ACA AAG ACG CCA GTC TTT GAT	204
	Ile Phe Ser Arg Arg Ala Thr Leu Asp Thr Lys Thr Pro Val Phe Asp	
	35 40 45	
	GTC GOC GAC GTG GAC AAG CAC GOC GAC GAC GTG GAC GTG CTG TTC CTG	252
20	Val Ala Asp Val Asp Lys His Ala Asp Asp Val Asp Val Leu Phe Leu	
	50 55 60	
	TGC ATG OGC TOC GOC ACC GAC ATC OCT GAG CAG GCA CCA AAG TTC GCG	300
	Cys Met Gly Ser Ala Thr Asp Ile Pro Glu Gln Ala Pro Lys Phe Ala	
	65 70 75 80	
25	CAG TTC OGC TGC ACC GTA GAC ACC TAC GAC AAC CAC CGC GAC ATC CCA	348
	Gln Phe Ala Cys Thr Val Asp Thr Tyr Asp Asn His Arg Asp Ile Pro	
	85 90 95	
	OGC CAC OGC CAG GTC ATG AAC GAA GOC GOC ACC GCA GOC GGC AAC GTT	396
30	Arg His Arg Gln Val Met Asn Glu Ala Ala Thr Ala Ala Gly Asn Val	
	100 105 110	
	GCA CTG GTC TCT ACC GGC TGG GAT CCA GGA ATG TTC TOC ATC AAC OGC	444
	Ala Leu Val Ser Thr Gly Trp Asp Pro Gly Met Phe Ser Ile Asn Arg	
	115 120 125	
35	GTC TAC GCA GOG GCA GTC TTA GOC GAG CAC CAG CAG CAC ACC TTC TGG	492
	Val Tyr Ala Ala Ala Val Leu Ala Glu His Gln Gln His Thr Phe Trp	
	130 135 140	
	GGC CCA GGT TTG TCA CAG GGC CAC TOC GAT GCT TTG CGA OGC ATC OCT	540
40	Gly Pro Gly Leu Ser Gln Gly His Ser Asp Ala Leu Arg Arg Ile Pro	
	145 150 155 160	
	GGC GTT CAA AAG GCA GTC CAG TAC ACC CTC CCA TOC GAA GAC GOC CTG	588
	Gly Val Gln Lys Ala Val Gln Tyr Thr Leu Pro Ser Glu Asp Ala Leu	
	165 170 175	
45	GAA AAG GOC OGC OGC GGC GAA GOC GGC GAC CTT AOC GGA AAG CAA ACC	636
	Glu Lys Ala Arg Arg Gly Glu Ala Gly Asp Leu Thr Gly Lys Gln Thr	
	180 185 190	
	CAC AAG OGC CAA TGC TTC GTG GTT GOC GAC GOG GOC GAT CAC GAG CGC	684
50	His Lys Arg Gln Cys Phe Val Val Ala Asp Ala Ala Asp His Glu Arg	
	195 200 205	

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	ATC GAA AAC GAC ATC CGC ACC ATG CCT GAT TAC TTC GTT GGC TAC GAA	732
	Ile Glu Asn Asp Ile Arg Thr Met Pro Asp Tyr Phe Val Gly Tyr Glu	
	210 215 220	
5	GTC GAA GTC AAC TTC ATC GAC GAA GCA ACC TTC GAC TCC GAG CAC ACC	780
	Val Glu Val Asn Phe Ile Asp Glu Ala Thr Phe Asp Ser Glu His Thr	
	225 230 235 240	
10	GGC ATG CCA CAC GGT GGC CAC GTG ATT ACC ACC GGC GAC ACC GGT GGC	828
	Gly Met Pro His Gly Gly His Val Ile Thr Thr Gly Asp Thr Gly Gly	
	245 250 255	
	TTC AAC CAC ACC GTG GAA TAC ATC CTC AAG CTG GAC CGA AAC CCA GAT	876
	Phe Asn His Thr Val Glu Tyr Ile Leu Lys Leu Asp Arg Asn Pro Asp	
	260 265 270	
15	TTC ACC GCT TCC TCA CAG ATC GCT TTC GGT CGC GCA GCT CAC CGC ATG	924
	Phe Thr Ala Ser Ser Gln Ile Ala Phe Gly Arg Ala Ala His Arg Met	
	275 280 285	
	AAG CAG CAG GGC CAA AGC GGA GCT TTC ACC GTC CTC GAA GTT GCT CCA	972
20	Lys Gln Gln Gly Gln Ser Gly Ala Phe Thr Val Leu Glu Val Ala Pro	
	290 295 300	
	TAC CTG CTC TCC CCA GAG AAC TTG GAC GAT CTG ATC GCA CGC GAC GTC	1020
	Tyr Leu Leu Ser Pro Glu Asn Leu Asp Asp Leu Ile Ala Arg Asp Val	
	305 310 315 320	
25	TAATTTAGCT CGAG	1034

## (2) INFORMATION FOR SEQ ID NO:24:

### (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 320 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

35	Met Thr Asn Ile Arg Val Ala Ile Val Gly Tyr Gly Asn Leu Gly Arg
	1 5 10 15
	Ser Val Glu Lys Leu Ile Ala Lys Gln Pro Asp Met Asp Leu Val Gly
	20 25 30
40	Ile Phe Ser Arg Arg Ala Thr Leu Asp Thr Lys Thr Pro Val Phe Asp
	35 40 45
	Val Ala Asp Val Asp Lys His Ala Asp Asp Val Asp Val Leu Phe Leu
	50 55 60
45	Cys Met Gly Ser Ala Thr Asp Ile Pro Glu Gln Ala Pro Lys Phe Ala
	65 70 75 80
	Gln Phe Ala Cys Thr Val Asp Thr Tyr Asp Asn His Arg Asp Ile Pro
	85 90 95
	Arg His Arg Gln Val Met Asn Glu Ala Ala Thr Ala Ala Gly Asn Val
50	100 105 110
	Ala Leu Val Ser Thr Gly Trp Asp Pro Gly Met Phe Ser Ile Asn Arg

115 120 125  
 Val Tyr Ala Ala Ala Val Leu Ala Glu His Gln Gln His Thr Phe Trp  
 130 135 140  
 Gly Pro Gly Leu Ser Gln Gly His Ser Asp Ala Leu Arg Arg Ile Pro  
 145 150 155 160  
 Gly Val Gln Lys Ala Val Gln Tyr Thr Leu Pro Ser Glu Asp Ala Leu  
 165 170 175  
 Glu Lys Ala Arg Arg Gly Glu Ala Gly Asp Leu Thr Gly Lys Gln Thr  
 180 185 190  
 His Lys Arg Gln Cys Phe Val Val Ala Asp Ala Ala Asp His Glu Arg  
 195 200 205  
 Ile Glu Asn Asp Ile Arg Thr Met Pro Asp Tyr Phe Val Gly Tyr Glu  
 210 215 220  
 Val Glu Val Asn Phe Ile Asp Glu Ala Thr Phe Asp Ser Glu His Thr  
 225 230 235 240  
 Gly Met Pro His Gly Gly His Val Ile Thr Thr Gly Asp Thr Gly Gly  
 245 250 255  
 Phe Asn His Thr Val Glu Tyr Ile Leu Lys Leu Asp Arg Asn Pro Asp  
 260 265 270  
 Phe Thr Ala Ser Ser Gln Ile Ala Phe Gly Arg Ala Ala His Arg Met  
 275 280 285  
 Lys Gln Gln Gly Gln Ser Gly Ala Phe Thr Val Leu Glu Val Ala Pro  
 290 295 300  
 Tyr Leu Leu Ser Pro Glu Asn Leu Asp Asp Leu Ile Ala Arg Asp Val  
 305 310 315 320

## Claims

1. A recombinant DNA autonomously replicable in cells of coryneform bacteria, comprising a DNA sequence coding for an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, and a DNA sequence coding for a dihydrodipicolinate reductase.
2. The recombinant DNA according to claim 1, further comprising a DNA sequence coding for a dihydrodipicolinate synthase.
3. The recombinant DNA according to claim 2, further comprising a DNA sequence coding for a diaminopimelate decarboxylase.
4. The recombinant DNA according to claim 3, further comprising a DNA sequence coding for a diaminopimelate dehydrogenase.
5. The recombinant DNA according to any one of claims 1 to 4, wherein said aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized is an aspartokinase originating from coryneform bacteria, and wherein said aspartokinase is provided as a mutant aspartokinase in which a 279th alanine residue as counted from its N-terminal is changed into an amino acid residue other than alanine and other than acidic amino acid in its  $\alpha$ -subunit, and a 30th alanine residue is changed into an amino acid residue other than alanine and other than acidic amino acid in its  $\beta$ -subunit.

6. The recombinant DNA according to any one of claims 1 to 4, wherein said DNA sequence coding for the dihydrodipicolinate reductase codes for an amino acid sequence depicted in SEQ ID NO: 15 in Sequence Listing, or an amino acid sequence substantially the same as the amino acid sequence depicted in SEQ ID NO: 15.
- 5 7. The recombinant DNA according to claim 2, wherein said DNA sequence coding for the dihydrodipicolinate synthase codes for an amino acid sequence depicted in SEQ ID NO: 11 in Sequence Listing, or an amino acid sequence substantially the same as the amino acid sequence depicted in SEQ ID NO: 11.
- 10 8. The recombinant DNA according to claim 3, wherein said DNA sequence coding for the diaminopimelate decarboxylase codes for an amino acid sequence depicted in SEQ ID NO: 19 in Sequence Listing, or an amino acid sequence substantially the same as the amino acid sequence depicted in SEQ ID NO: 19.
- 15 9. The recombinant DNA according to claim 4, wherein said DNA sequence coding for the diaminopimelate dehydrogenase codes for an amino acid sequence depicted in SEQ ID NO: 24 in Sequence Listing, or an amino acid sequence substantially the same as the amino acid sequence depicted in SEQ ID NO: 24.
- 20 10. A coryneform bacterium harboring an aspartokinase in which feedback inhibition by L-lysine and L-threonine is substantially desensitized, and comprising an enhanced DNA sequence coding for a dihydrodipicolinate reductase.
- 25 11. The coryneform bacterium according to claim 10, transformed by introduction of the recombinant DNA as defined in claim 1.
- 30 12. The coryneform bacterium according to claim 10, further comprising an enhanced DNA sequence coding for a dihydrodipicolinate synthase.
- 35 13. The coryneform bacterium according to claim 12, transformed by introduction of the recombinant DNA as defined in claim 2.
- 40 14. The coryneform bacterium according to claim 12, further comprising an enhanced DNA sequence coding for a diaminopimelate decarboxylase.
- 45 15. The coryneform bacterium according to claim 14, transformed by introduction of the recombinant DNA as defined in claim 3.
- 50 16. The coryneform bacterium according to claim 14, further comprising an enhanced DNA sequence coding for a diaminopimelate dehydrogenase.
- 55 17. The coryneform bacterium according to claim 16, transformed by introduction of the recombinant DNA as defined in claim 4.
18. A method for producing L-lysine comprising the steps of cultivating said coryneform bacterium as defined in any one of claims 10 to 17 in an appropriate medium, producing and accumulating L-lysine in a culture of the bacterium, and collecting L-lysine from the culture.

FIG. 1

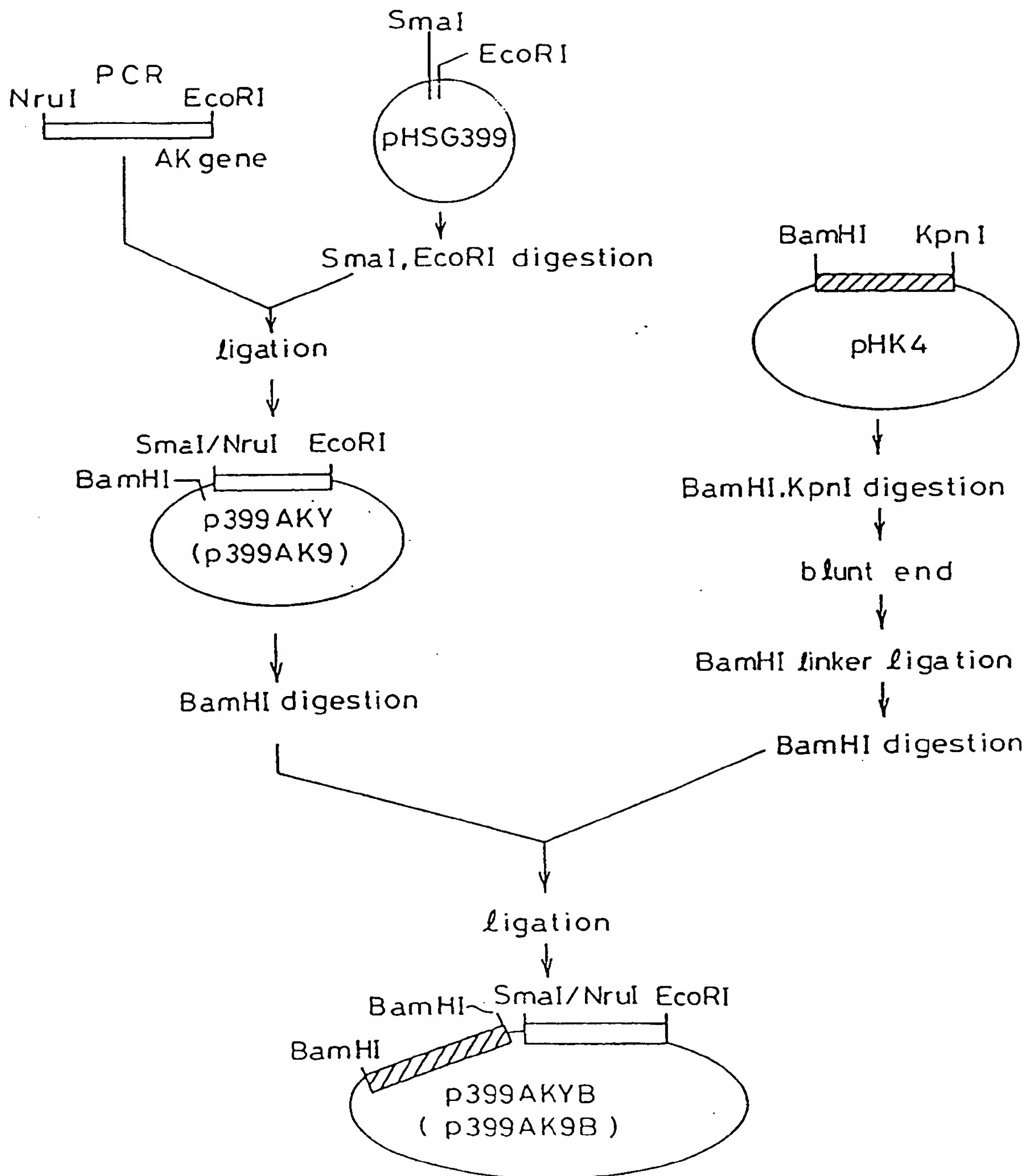




FIG. 2

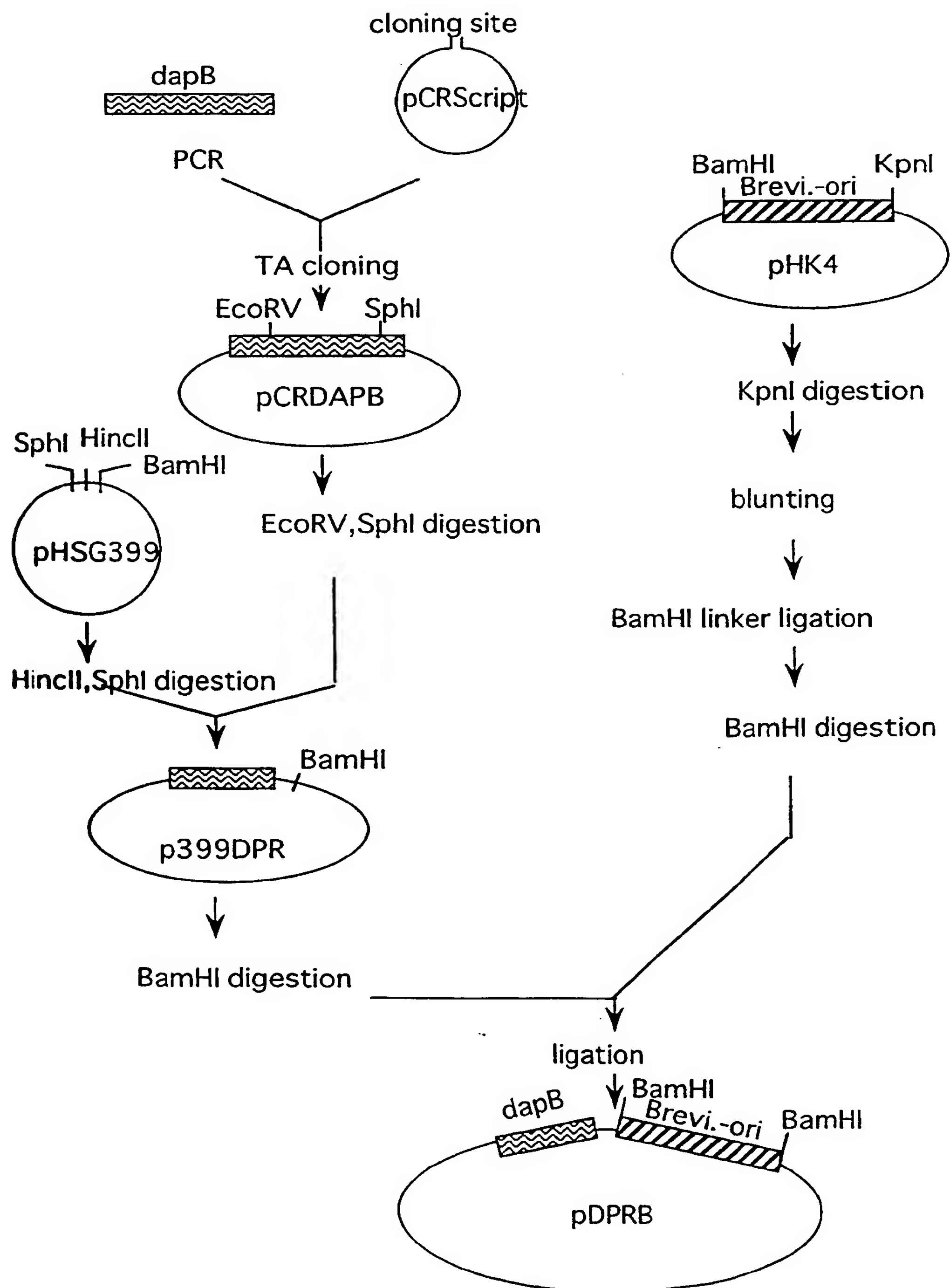


FIG. 3

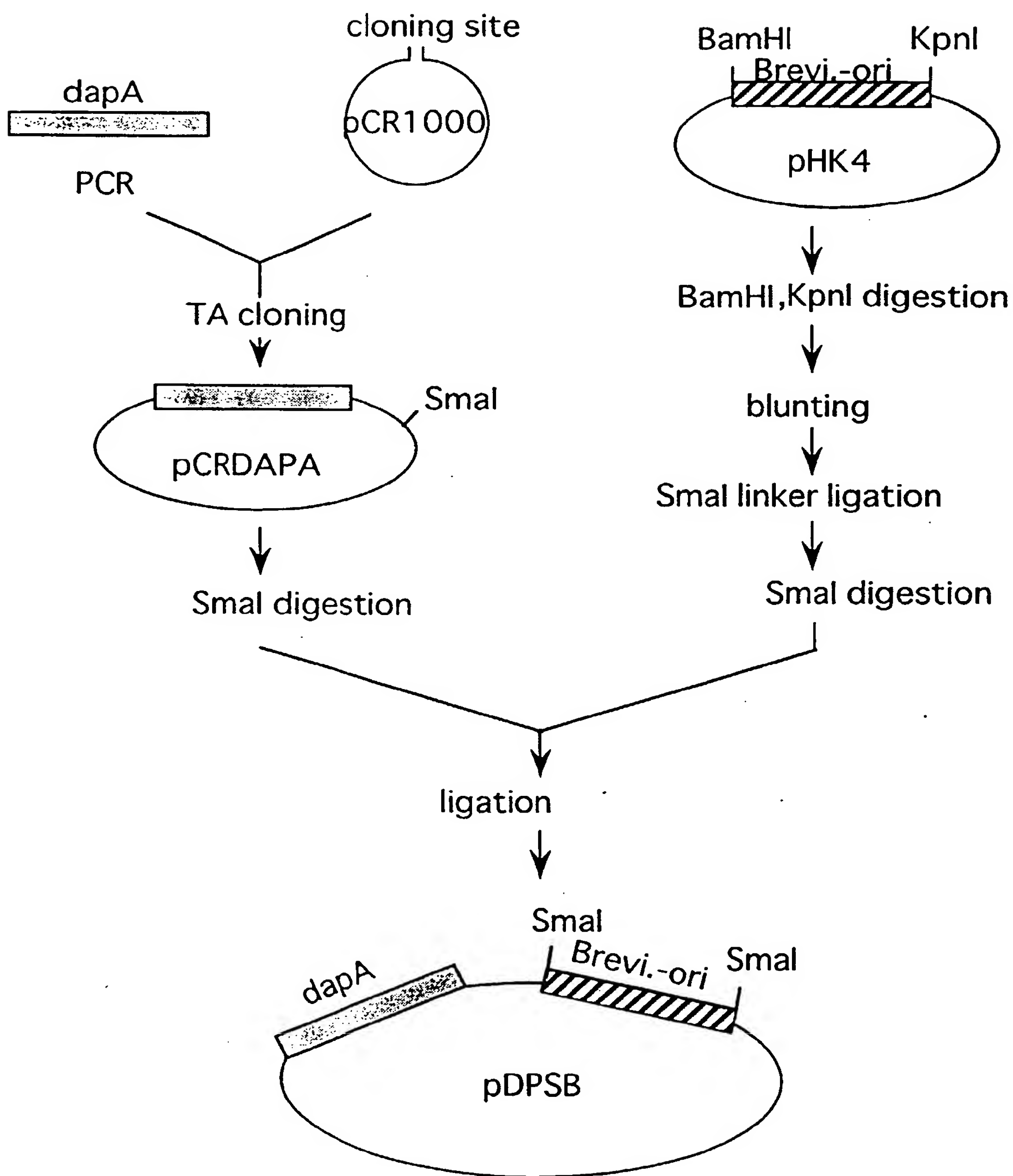


FIG. 4

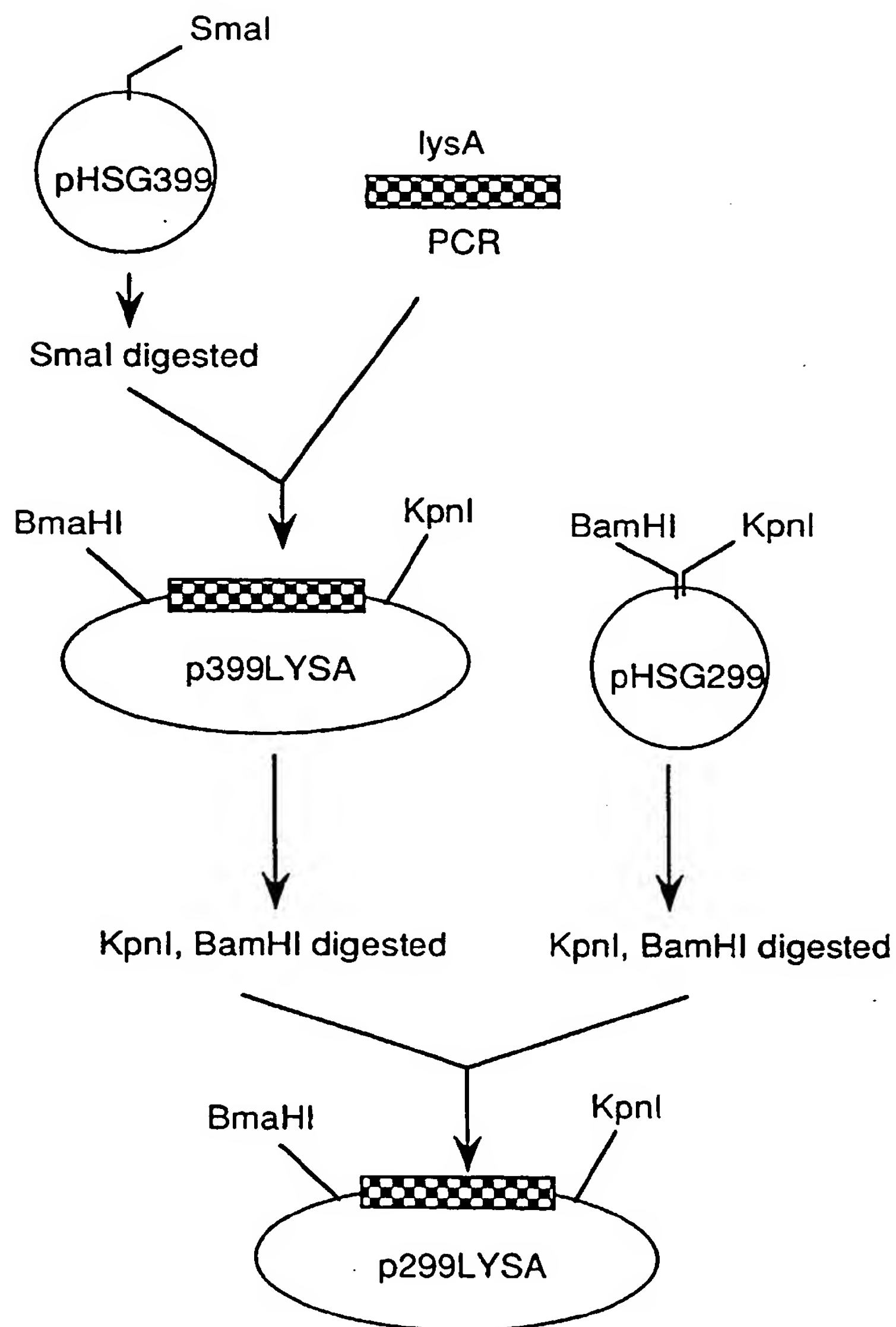


FIG. 5

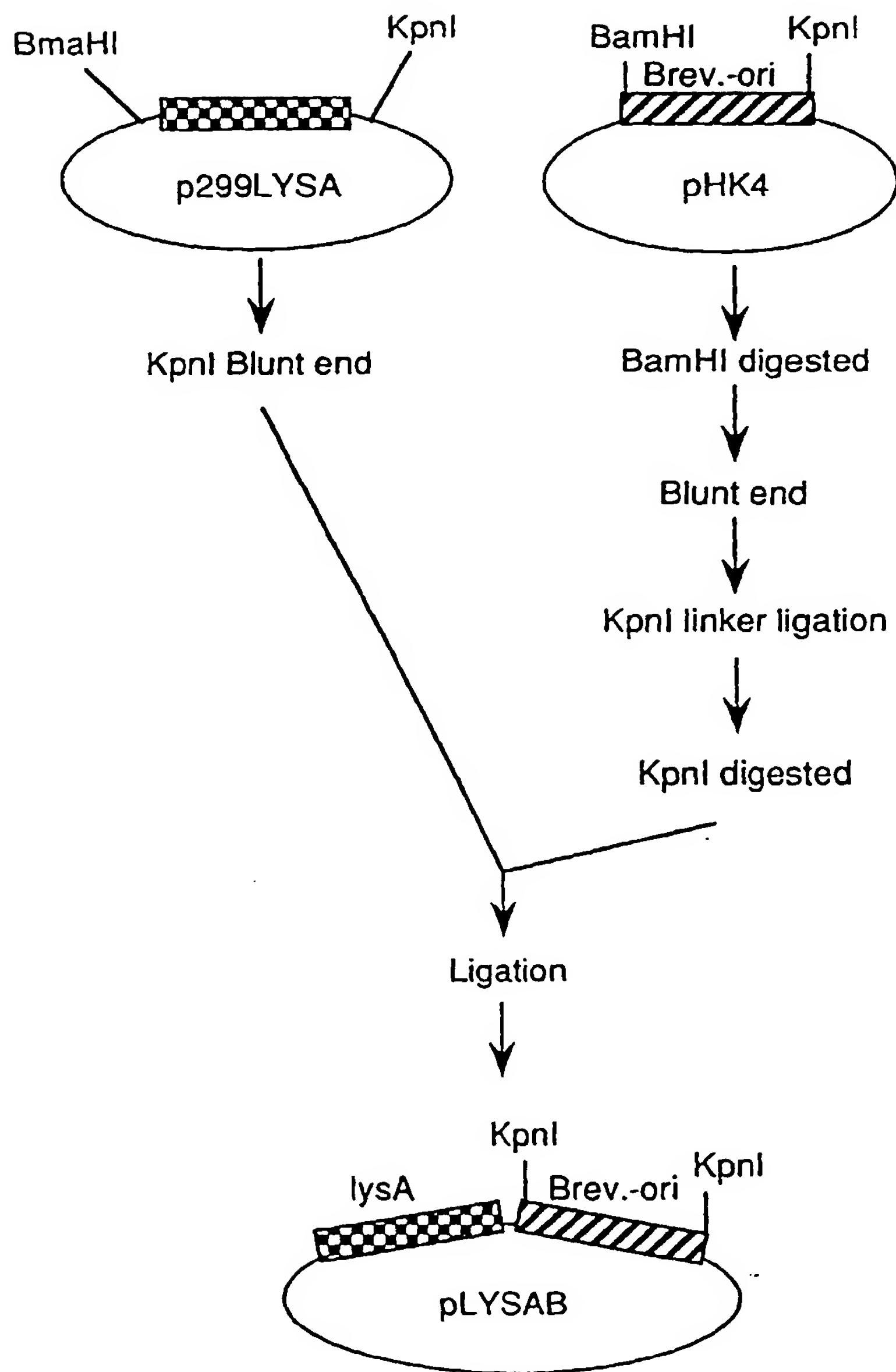


FIG. 6

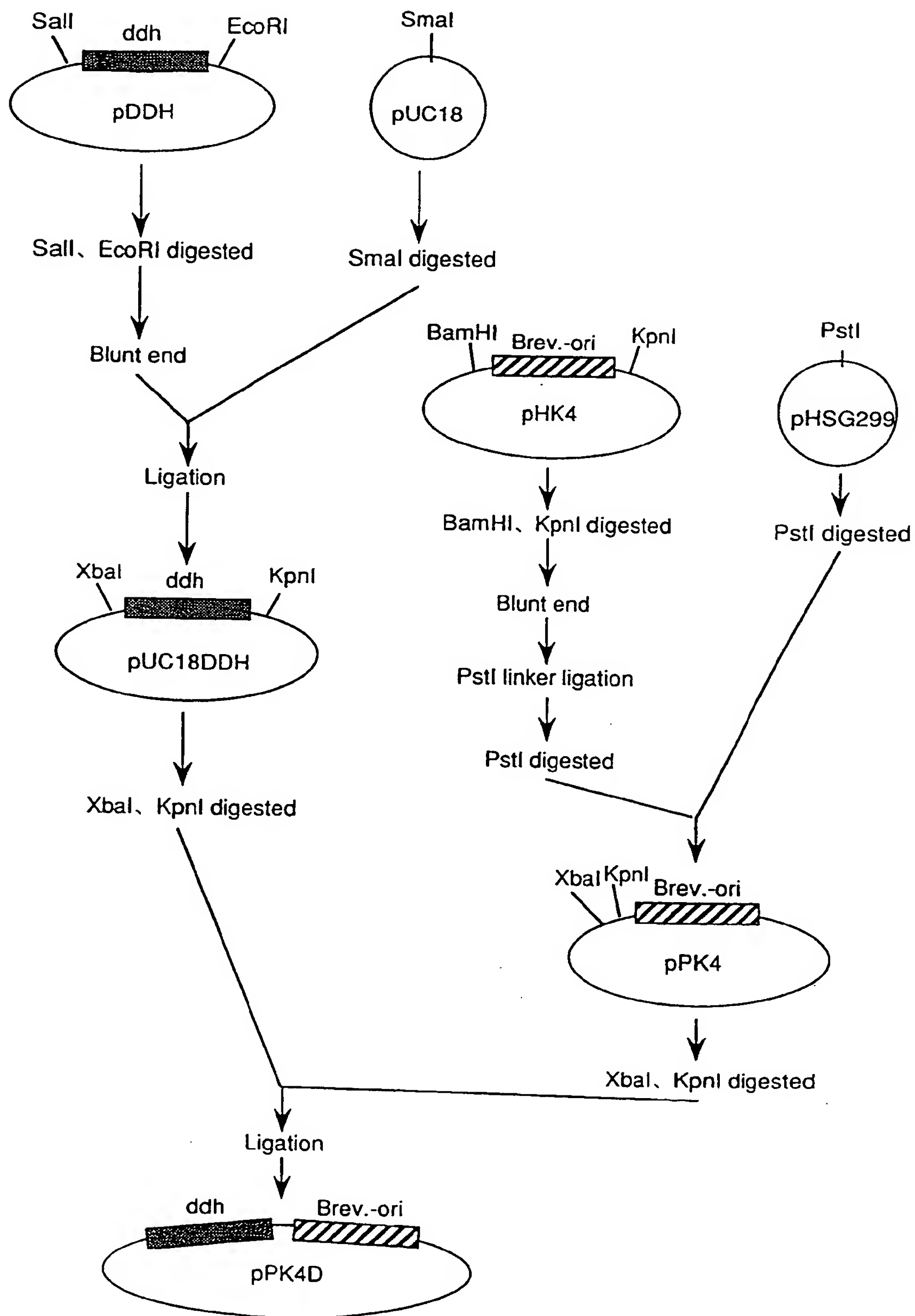




FIG. 7

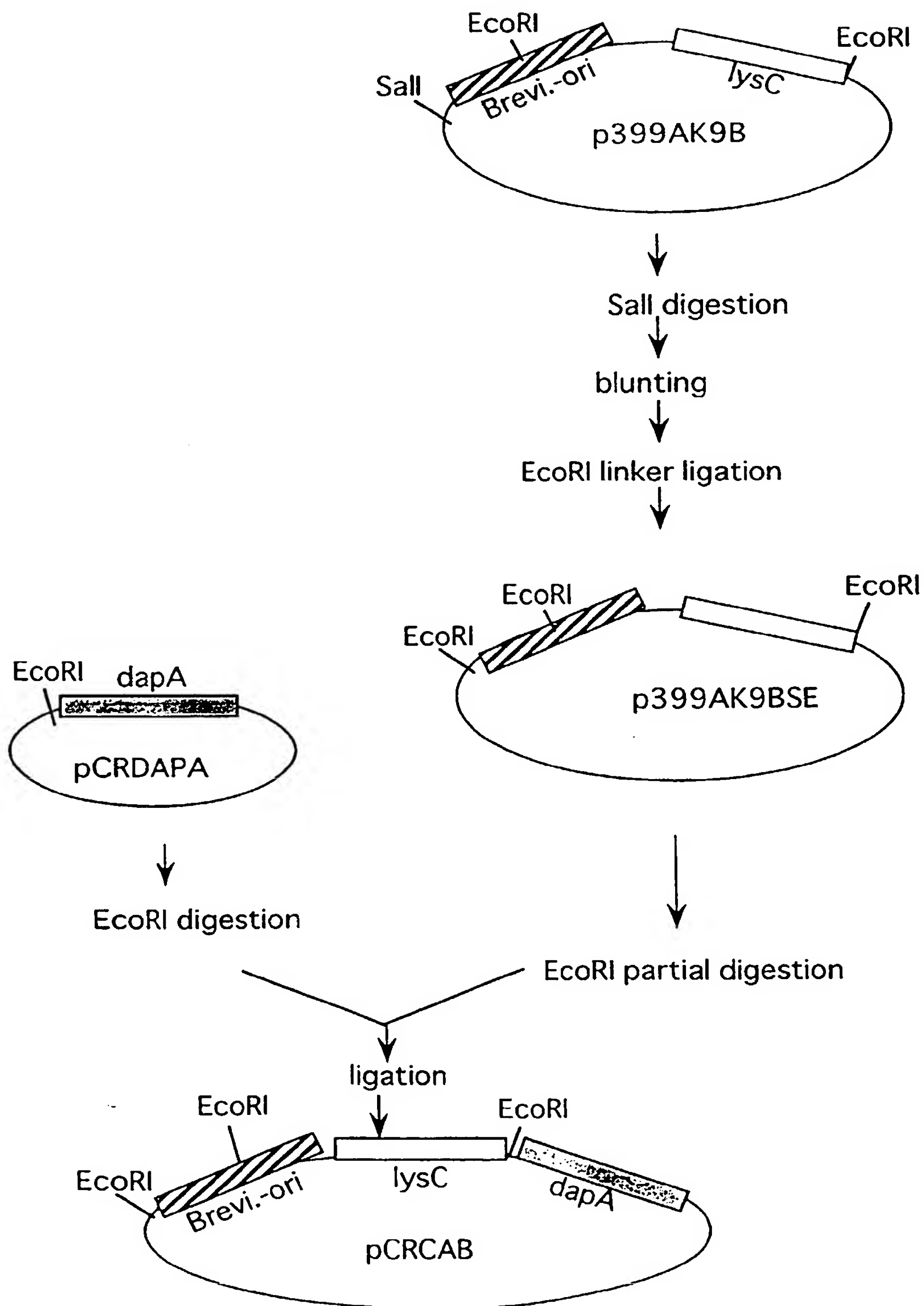


FIG. 8

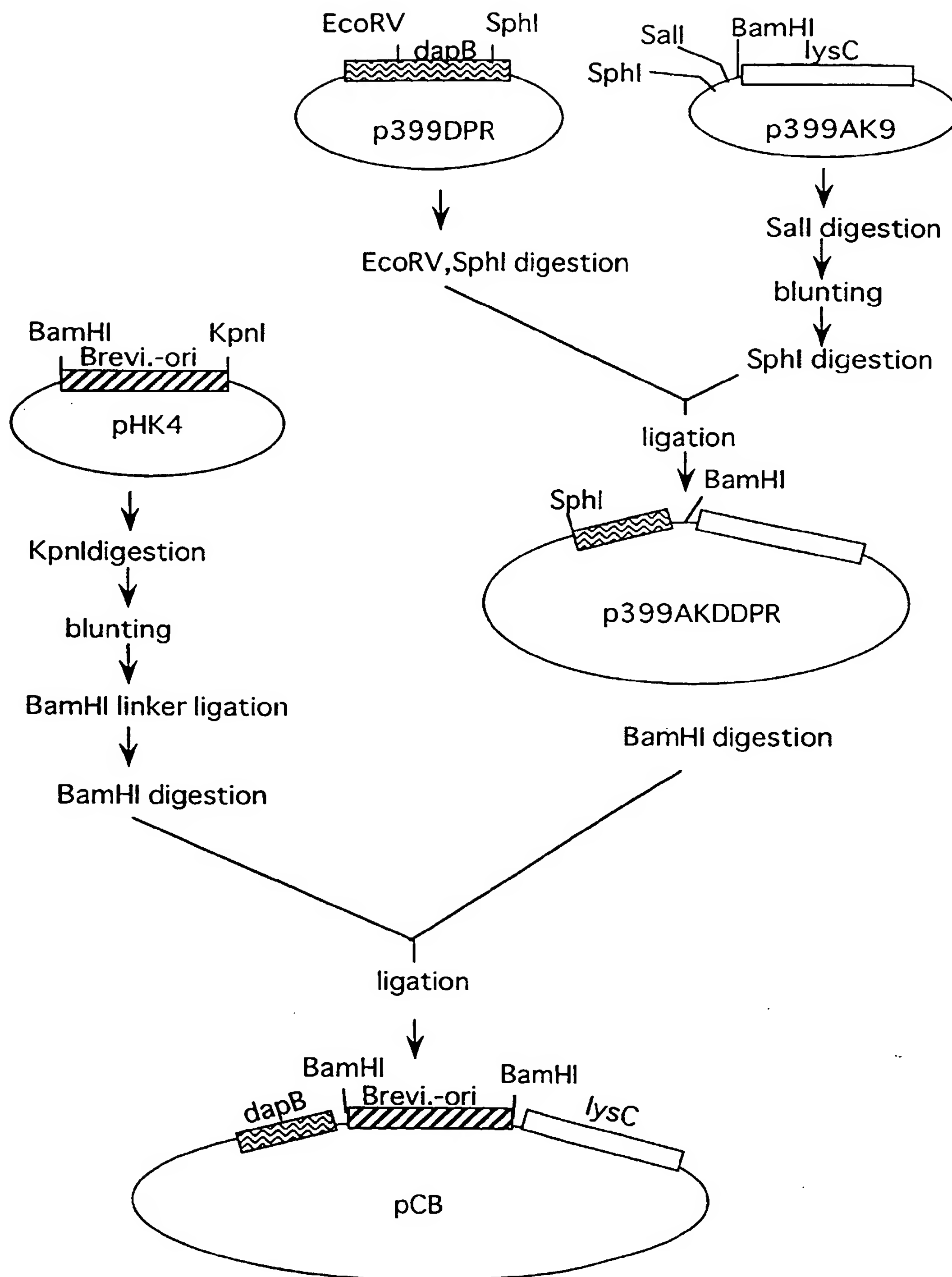


FIG. 9

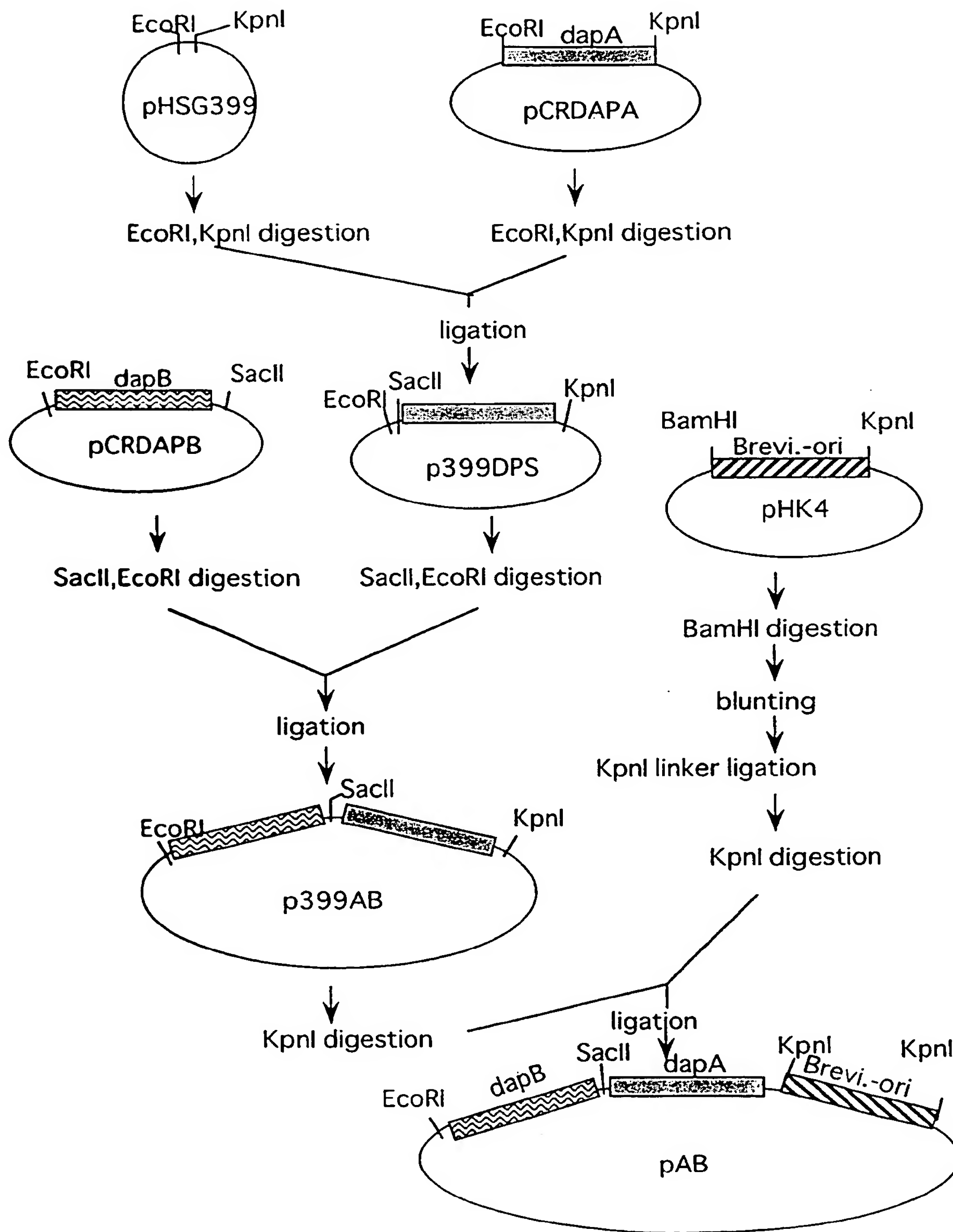


FIG. 10

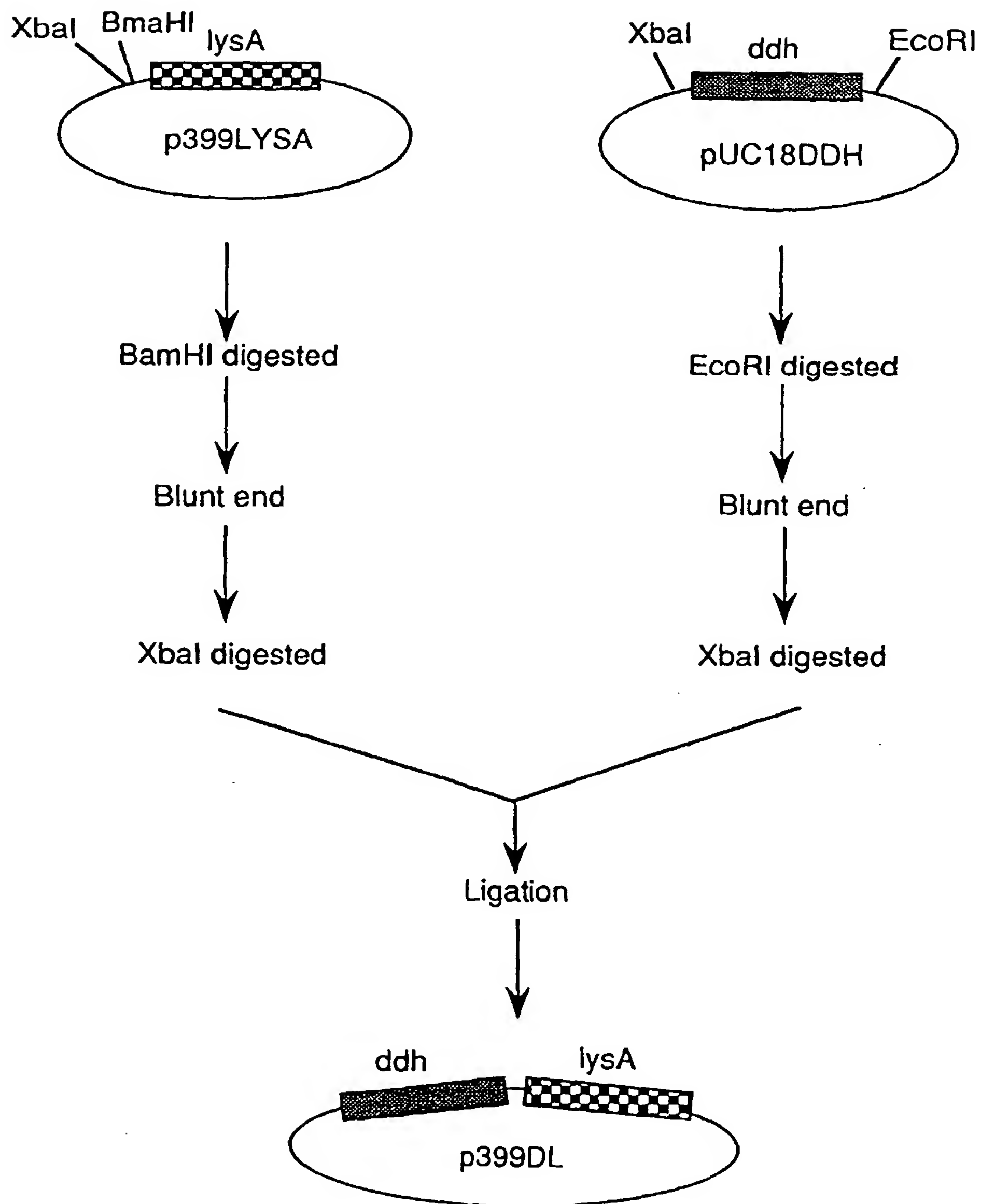


FIG. 11

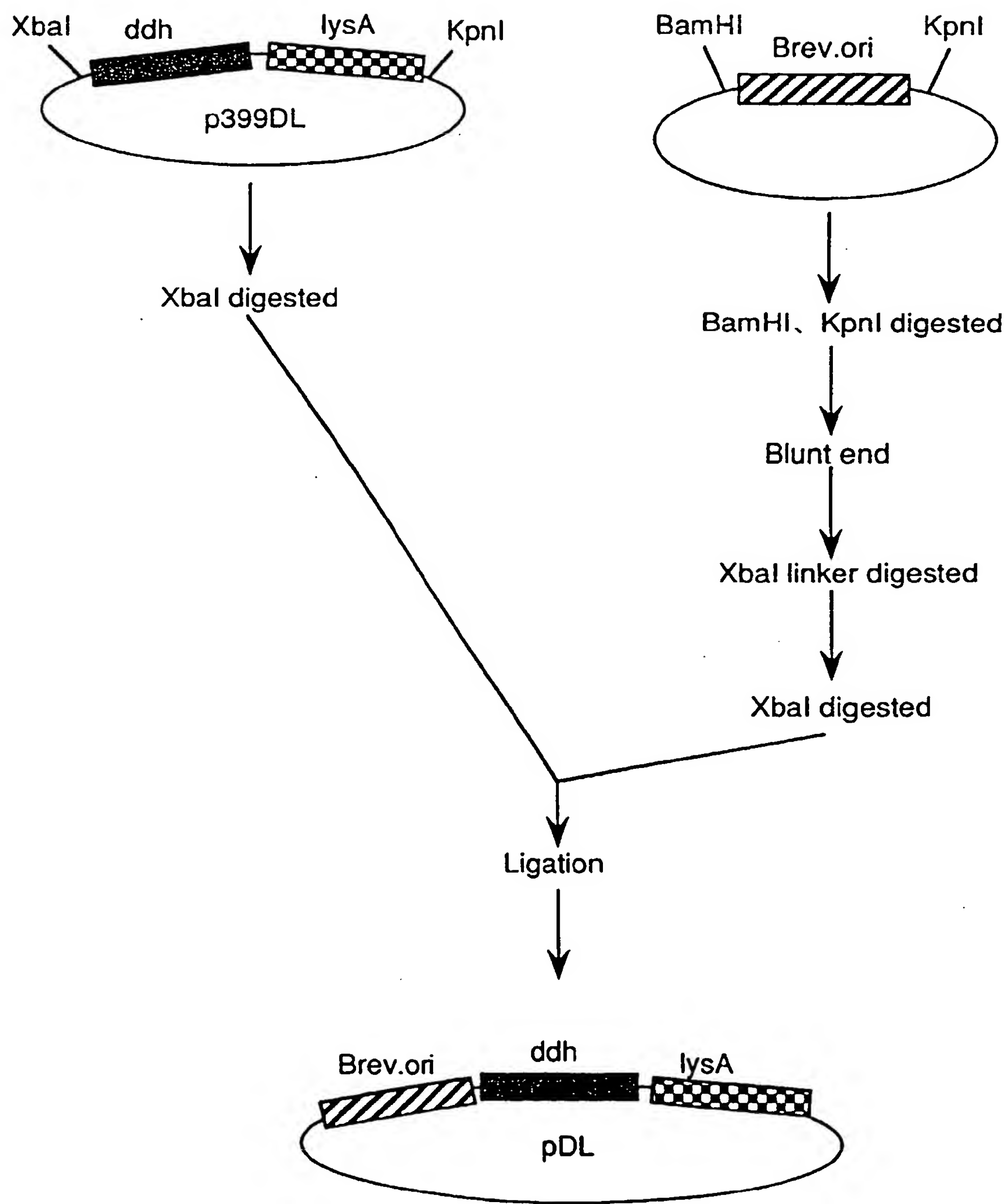




FIG. 12

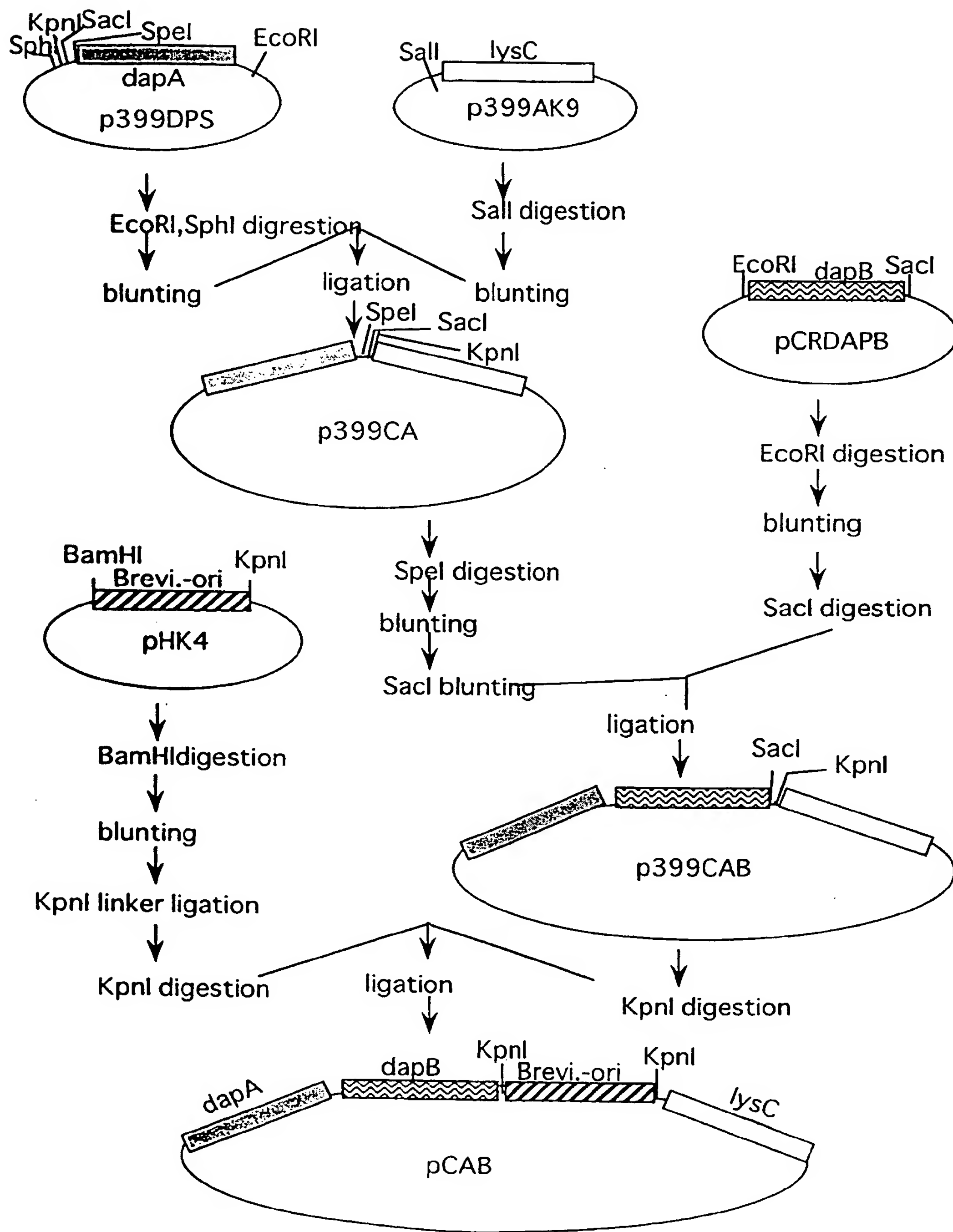


FIG. 13

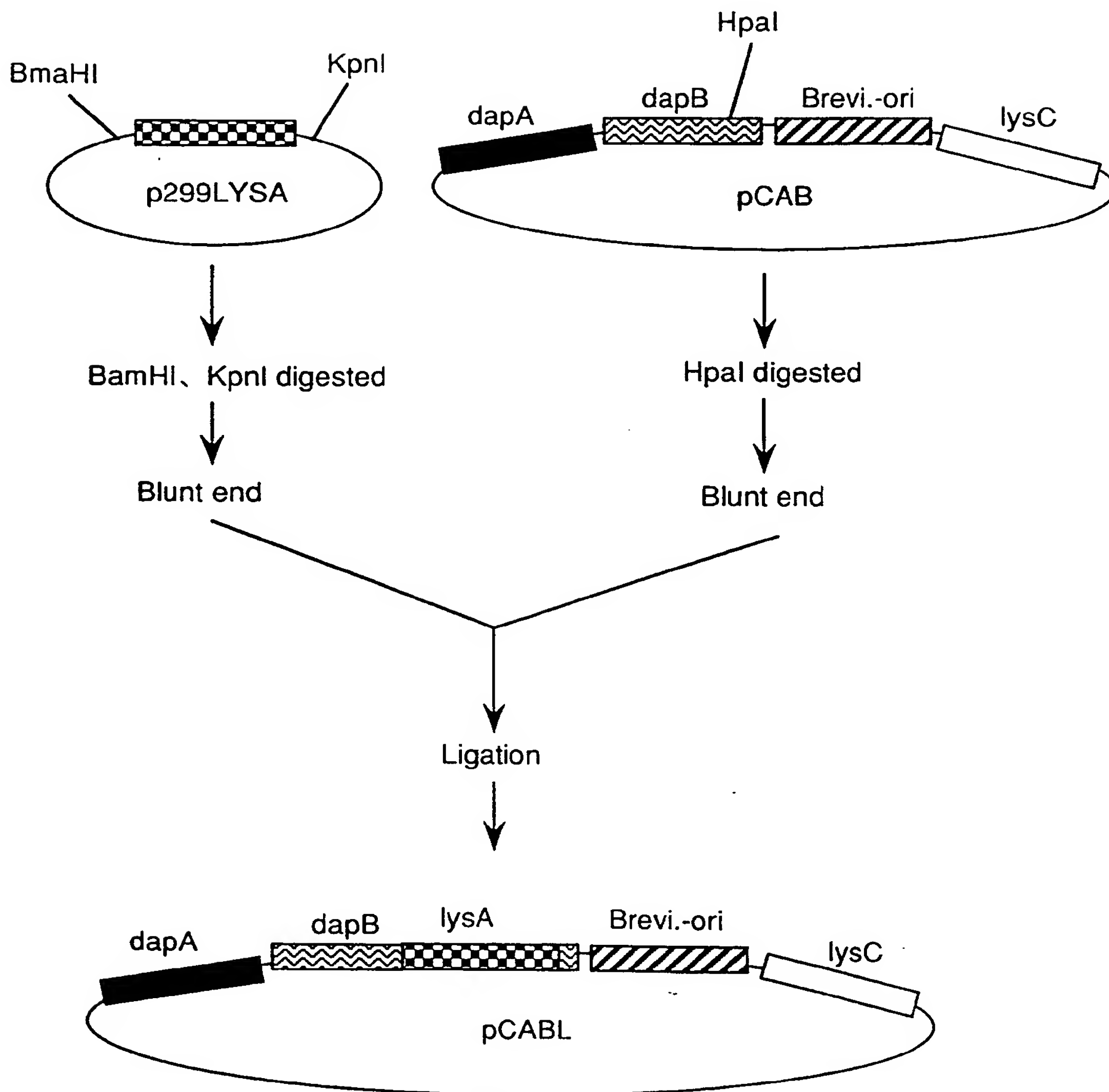
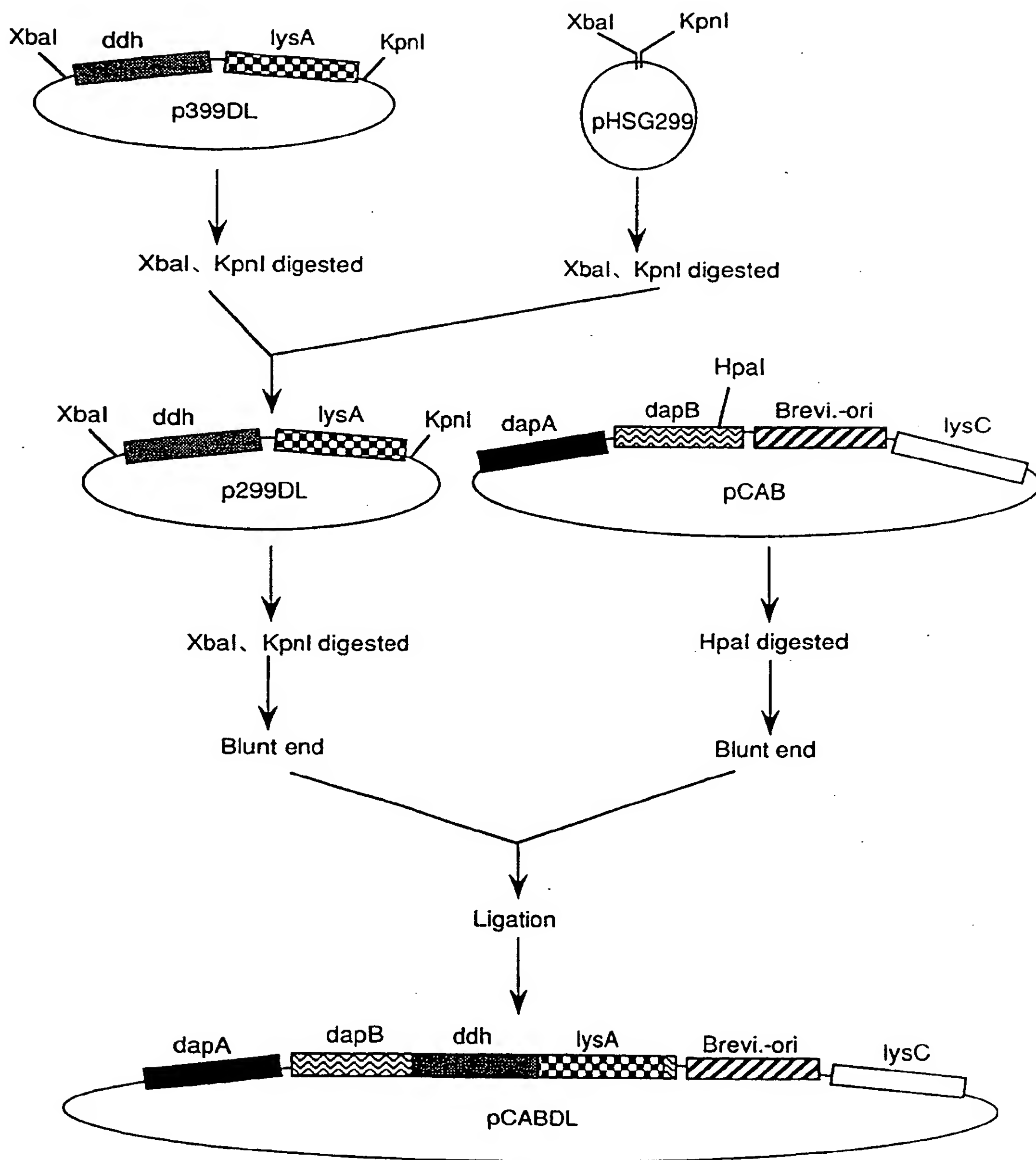


FIG. 14



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP96/01511

## A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl<sup>6</sup> C12N15/52, C12N1/21, C12P13/08 // (C12N15/52, C12R1:13),  
(C12N1/21, C12R1:13), (C12P13/08, C12R1:13)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl<sup>6</sup> C12N15/52, C12N1/21, C12P13/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, BIOSIS PREVIEWS, WPI/L

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y/A	Applied and Environmental Microbiology, vol. 57, No. 6 (1991), Hermann Sahm, et al., see p. 1746-1752	1-18/5
Y	JP, 7-75578, A (Mitsubishi Petrochemical Co., Ltd.), March 20, 1995 (20. 03. 95) (Family: none)	1 - 18
Y	Nucleic Acids Res., Vol. 15, No. 9, (1987), Kazumi Araki, et al., see p. 3917	4, 9, 16-18
Y	Molecular Microbiology, Vol. 4, No. 11, (1990), A. J. Sinskey, et al., see p. 1819-1830	3, 8, 14-15, 18
Y	Molecular and General Genetics, Vol. 212, No. 1, (1988), A. J. Sinskey, et al., see p. 112-119	3, 8, 14-15, 18

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

August 30, 1996 (30. 08. 96)

Date of mailing of the international search report

September 10, 1996 (10. 09. 96)

Name and mailing address of the ISA/

Japanese Patent Office

Facsimile No.

Authorized officer

Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

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